

Diagnostic Usefulness and Limitations of the Sural Nerve Biopsy

Shin J. Oh

In recent years, the sural nerve biopsy has become a commonly performed procedure in the diagnostic work-up of patients with peripheral neuropathy. This paper reviews the diagnostic usefulness and limitations of this procedure. Based on 385 sural nerve biopsies, we found clinically helpful or relevant information in 45% of cases. In 24% of cases, specific diagnoses were obtained, among which vasculitic neuropathy was most common.

Key Words: nerve biopsy, sural nerve biopsy, peripheral neuropathy, diagnosis of neuropathy

In the past decade, the sural nerve biopsy has become a commonly applied procedure in the diagnostic work-up in patients with peripheral neuropathy. This is mainly due to the increasing awareness among clinicians that the nerve biopsy is helpful in identifying treatable causes of neuropathy. This paper will discuss the usefulness and limitations of the sural nerve biopsy.

CLASSIFICATION OF PERIPHERAL NEUROPATHY

There are two major anatomic components of the peripheral nerve, axons and myelin. Peripheral nerve axons are simply cytoplasmic extensions of the neurons. The axons are responsible for the maintenance and function of the peripheral nerves and derive most of the protein essential for this purpose from the neurons. Along the axons, membrane components, organelles, nutrients, and metabolic products are transported as axoplasm at different velocities in both directions (Schwartz 1979). This system renders the axons exquisitely vulnerable to any metabolic changes in the neurons. The myelin of peripheral nerve is derived from the Schwann cells and is depen-

dent both on the Schwann cells themselves and the axons for its continued integrity. Myelin is responsible for the conduction of nerve action potentials along the nerves. This is due to "saltatory conduction" in myelinated fibers. Schwann cells envelop axons to form unmyelinated and myelinated fibers surrounded by basal lamina. A single Schwann cell occupies each myelinated internode, almost never associating itself with more than one axon (Berthold 1978).

Depending upon which components of the peripheral nerve are predominantly involved in the pathological process, peripheral neuropathy can be classified into two main categories: axonal neuropathy due to axonal degeneration and demyelinating neuropathy due to segmental demyelination. There are also clear pathophysiological differences between axonal degeneration and segmental demyelination, as noted in Table 1 (Oh 1983).

In axonal neuropathy, the disease process affects axons primarily producing axonal degeneration and secondarily causing breakdown of the myelin sheath (Fig. 1). Axonal degeneration is induced either by a metabolic derangement in the neuron cell body (neuronopathy) or throughout the axon (dying-back axonal degeneration). Severe damage to the neurons and disruption of proximal axonal integrity results in rapid degeneration of the entire distal portion of axons. The myelin sheath breaks down concomitantly with the axon. The clinical effect of this phenomenon is that of a distal symmetrical polyneuropathy. Nerve conduction in axonal neuropathy is either normal or mildly slow though the amplitudes of the compound muscle action potential (CMAP) and compound nerve

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Department of Neurology, The University of Alabama at Birmingham, The Veterans Administration Medical Center Birmingham, Alabama, 35294, USA

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Address: reprint requests to Dr. S J Oh, Department of Neurology, The University of Alabama at Birmingham Medical Center UAB station, Birmingham, Alabama, 35294, U.S.A.

Table 1. Pathophysiology of two types of peripheral neuropathy

Type	Axonal neuropathy	Demyelinating neuropathy
Primary lesion	Axon	Myelin
Pathological process	Axonal degeneration	Demyelination
Pathology by teasing preparation	Myelin ovoids	Segmental demyelination
Regeneration:		
mechanism	Axonal sprouting	Remyelination
speed	Slow	Rapid
Nerve conduction:		
velocity	Mildly slow; above 30 m/sec	Markedly slow; below 30 m/sec
CMAP	Low amplitude	Dispersion; conduction block
Needle EMG:		
Fibrillation & positive sharp wave	(++++)	(-) or (±)
Fasciculation	Absent	Present in chronic form
Examples:*	Arsenic	Guillain-Barre syndrome
	Alcoholic	CIDP
	Nutritional	Hypertrophic
	Vasculitic	Metachromatic
	Giant axonal	Tomaculous
	Thallium	Leprosy
	Vitamine B12	Hypothyroid
	Gold	Diphtheric

* Diabetic and uremic neuropathies are most likely due to the combined processes of axonal degeneration and demyelination.

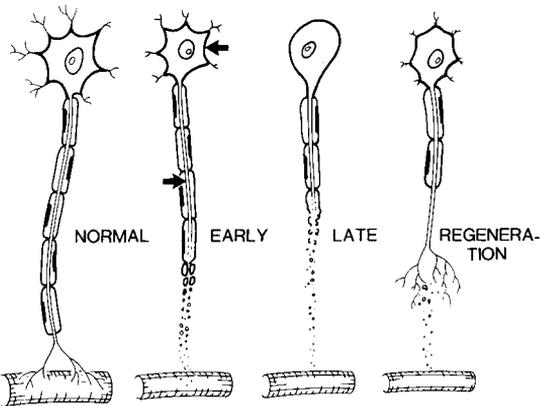


Fig. 1. Mechanism of axonal degeneration and regeneration. Axonal degeneration is induced either by a metabolic derangement in the neuron cell body (neuronopathy) or throughout the axon (dying-back axonal degeneration) (early; arrows). Damage to the neurons and disruption of proximal axonal integrity result in rapid degeneration of the entire distal portion of axon, producing breakdown of the myelin sheath (late). Regeneration occurs with axonal sprouting.

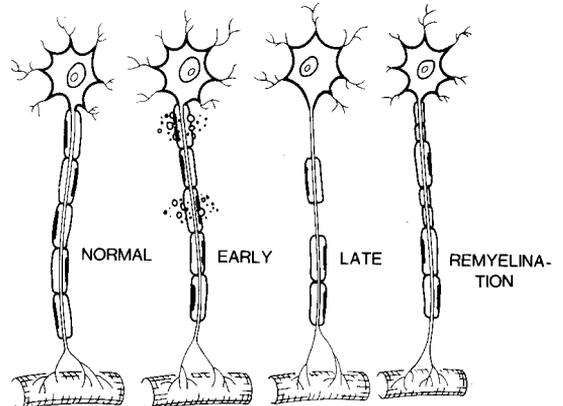


Fig. 2. Mechanism of segmental demyelination and remyelination. Segmental demyelination is induced by metabolic damage of Schwann cells or peeling and engulfment by activated inflammatory cells (early). This process affects the myelin sheath producing primary segmental demyelination and leaving the axon intact (late). Remyelination occurs with myelination over demyelinated segment.

Table 2. Frequency of unknown causes in peripheral neuropathy

Authors	Case number	Unknown cause (%)	Comments
Matthews (1952)	46	70	GBS is listed as an unknown cause
Rose (1960)	80	56	GBS is listed as a known cause
Prineas (1970)	278	14	GBS is listed as a known cause
Dyck (1981)	205	24	Inflammatory neuropathy is listed as a known cause
Faguis (1983)	91	74	Chronic inflammatory or hereditary neuropathies as unknown causes
McLeod (1984)	519	13	Inflammatory neuropathy is listed as a known cause. All cases had sural nerve biopsy

action potential (CNAP) are markedly reduced. Profuse fibrillation and positive sharp waves are prominent features in the needle EMG. Recovery occurs by the process of axonal sprouting and is slow, at a rate of 2 or 3mm per day, depending on the nature of the neuropathy and its severity. Many metabolic and toxic neuropathies are considered to be due to axonal degeneration (Table 1). Characteristically, the neuropathy is insidious in onset, commences distally, and slowly proceeds proximally, resulting in symmetrical distal polyneuropathy.

In demyelinating neuropathy, the disease process affects the myelin sheath producing primary segmental demyelination and leaving the axon intact (Fig. 2). Segmental demyelination is induced by metabolic damage of Schwann cells or peeling and engulfment by activated inflammatory cells. Nerve conduction in demyelinating neuropathy is characterized by marked slowing or conduction block. Fibrillation or positive sharp waves are either absent or rare in the needle EMG. Recovery occurs by the process of remyelination of the shorter intersegments on the demyelinated segment. Once remyelination begins, recovery is usually rapid and complete. The majority of immune mediated neuropathies such as GBS or chronic inflammatory neuropathy are considered to be due to segmental demyelination (Table 1). Characteristically, the neuropathy is diffuse and predominantly motor and the spinal fluid protein is elevated.

CAUSES FOR PERIPHERAL NEUROPATHY

The most common known cause of peripheral neuropathy is diabetes mellitus in the United States, while in the world as a whole it is leprosy (Bradley 1981). For neurology patients, the most common cause of peripheral neuropathy is the Guillain-Barre syndrome.

Despite extensive and costly evaluations, the causes of peripheral neuropathy remain unknown in a substantial number of cases. In some recent studies, the causes have not been determined in only 13-24% of cases (Table 2) (Dyck and Oviatt 1981; McLeod et al. 1984). These two low figures were reported from centers where the sural nerve biopsy is extensively used in identifying the cause of peripheral neuropathy. Compared with data in 1960, the frequency of unknown causes has decreased over the years. This decrease is mainly due to four factors: (1) greater sophistication of the electrophysiological study in differentiation between axonal neuropathy and demyelinating neuropathy; (2) inflammatory neuropathy such as the Guillain-Barre syndrome (GBS) is now classified as "known" in causes; (3) monoclonal gammopathy and paraneoplastic neuropathy are now known causes of some neuropathies, and (4) increasing use of the nerve biopsy in the work-up for peripheral neuropathy.

INDICATION FOR THE NERVE BIOPSY

If the cause of the neuropathy is known by the means of clinical examination and laboratory tests, the nerve biopsy is not indicated. In many metabolic neuropathies, history and laboratory tests are enough to make a definite causative diagnosis. These include diabetic, alcoholic, and uremic neuropathies. In these patients, the nerve biopsy is performed only to study the basic pathophysiology of neuropathy. Even for the GBS, the most common form of neuropathy for neurologists, the nerve biopsy is not indicated simply because the diagnosis can be made with certainty in most cases on the basis of the clinical, electrophysiological and spinal fluid findings.

The nerve biopsy is clearly indicated in two groups of patients: patients suspected of vasculitis and pa-

Table 3. Diagnostic usefulness of the sural nerve biopsy

Total number of cases: 385 cases at the UAB	
Specific diagnoses	24%
Chronic inflammatory demyelinating polyneuropathy	12%
Hereditary neuropathy	9%
Total percentage	45%

Table 4. Specific diagnoses among 385 cases at the UAB

Vasculitic neuropathy	12%
Hypertrophic neuropathy	7%
Inflammatory neuropathy	3%
Ischemic neuropathy	0.8%
Amyloid neuropathy	0.5%
Metachromatic neuropathy	0.3%
Sarcoid neuropathy	0.3%

tients with clinically significant peripheral neuropathy without known cause. The sural nerve biopsy is best indicated in patients suspected of having vasculitis, with or without the clinical features of neuropathy (Wees *et al.* 1981). This is because the nerve is more commonly involved than other readily available biopsied tissues such as skin and muscle and the diagnostic yield of the sural nerve biopsy is high in vasculitis (Wees *et al.* 1981). Peripheral neuropathy was reported in 52-60% of patients with vasculitis (Frohnert and Sheps 1967; Cohen *et al.* 1980). The nerve conduction test was crucial in these patients because it detected neuropathy in asymptomatic patients and because vasculitis was invariably found in the sural nerve when the nerve conduction was abnormal. The reason for indication of the sural nerve biopsy in neuropathy without known causes is obvious, because a definite diagnosis and other clinically helpful information can be obtained in some patients.

Based on data obtained in 385 sural nerve biopsies performed over a 16-year-period (1971-1986), we found clinically helpful or relevant information in 45% of case (Table 3). Specific diagnoses were obtained in 24% of cases; diagnosis of subacute or chronic inflammatory neuropathy was confirmed in 12% and hereditary neuropathy was diagnosed in 9% of cases. Among the specific diagnoses, vasculitic neuropathy was the most common form of neuropathy, accounting for 12% of 385 nerve biopsies (Table 4). Once a specific diagnosis is made, it dictates the clinical management of the disorder. This is best exemplified in vasculitic neuropathy where steroid and cytotoxic

agents are very helpful in inducing remission (Fauci *et al.* 1979). In subacute or chronic inflammatory neuropathy, long-term steroid treatment, often for many years, is required (Oh 1978). Thus, it is essential to confirm such diagnoses by the nerve biopsy before steroid is administered. Confirmation of hereditary neuropathy is helpful in predicting the progression of disease and in genetic counselling of patients. But, the nerve biopsy was not clinically helpful in 55% of cases.

Diagnostic sensitivity of the sural nerve biopsy is analysed in Table 5. It is important to recognize that, in only 24% of cases, were specific diagnoses made. In 55% of cases, the diagnosis of demyelinating or axonal neuropathy was made without further elucidation of any specific cause. In these cases, the nerve biopsy findings have to be correlated with the clinical information to reach a final diagnosis. This underlines the importance of exhaustive and detailed clinical examinations in the work-up of neuropathy.

THE SURAL NERVE BIOPSY

Biopsy of three different nerve have been described: radial sensory nerve, superficial peroneal nerve, and sural nerve. We prefer the sural nerve for biopsy for four reasons: (1) the nerve is easily identifiable and relatively protected from compression injury because it is located behind the lateral malleolus; (2) this nerve is purely sensory, thus producing no motor deficit following biopsy; (3) this nerve is liable to be affected by neuropathy because it is a distal branch of a long nerve; and (4) this nerve is easily tested electrophysiologically. We do not recommend the sural nerve biopsy if the nerve conduction is completely normal because the diagnostic yield is small. This policy is based on our experience in a few cases in which normal nerve biopsy was found when the nerve conduction was normal in the sural nerve. One disadvantage of this nerve biopsy is that the sural nerve is not affected if the neuropathy is purely motor. However, in practice, this does not impose a major problem because sensory nerve conduction is often

Table 5. Diagnostic sensitivity of the sural nerve biopsy

Total number of cases: 385 over 16 years at the UAB	
Specific diagnoses	24%
Demyelinating neuropathies	32%
Axonal neuropathies	23%
Non-specific findings	16%
Normal	5%

affected even in a clinically identified motor neuropathy such as GBS (Oh 1984).

The patient is placed in a lateral decubitus position and a pillow is placed under the ankle to be biopsied. The skin incision is begun under local anesthesia with 1% lidocaine behind the lateral malleolus and halfway between the posterior aspect of the Achilles tendon and the lateral malleolus. This incision is extended proximally for 4-5cm, parallel to the Achilles tendon. The whitish pearly sural nerve is identified medially under the lesser saphenous veins. When the sural nerve is touched by an instrument, often the patient feels a shooting electrical pain. Both are superficial to the deep fascia. If the sural nerve is not found easily, usually the examiner has gone too deep. Sometimes a lesser saphenous vein is mistakenly identified as a sural nerve. This can be avoided by carefully inspecting the nerve prior to cutting it, observing the broad angles at which the vein branches, in contrast to the narrow angles at which the nerve branches (Asbury and Connolly 1973). If a vein is cut, there is a tiny hole in the specimen. If the patient feels a shooting electrical pain when the nerve is transected, this confirms that the sural nerve has been cut. Once the sural nerve is identified, the nerve is anesthetized with a small amount of lidocaine a few millimeters proximal to the intended transection site prior to cutting in order to reduce the pain at time of nerve cutting. The patient should be warned that there will be a sharp pain at the moment the nerve is cut. This will improve a cooperation from the patient. Generally the degree of pain is inversely proportional to the severity of the neuropathy (Johnson 1985). The proximal nerve is pulled gently and cut with sharp dissection as high up in the incision as possible so that the cut end of the nerve will retract out of the operative field, resulting in a reduced incidence of subsequent traumatic neuroma formation (Johnson 1985). At least 3cm length of nerve should be obtained with due care in order to avoid any unnecessary trauma on the nerve. The skin incision is closed using interrupted mattress skin sutures with 4-0 coated vicryl suture inside

Table 6. Serious side-reaction of the nerve biopsy

Total Number of cases: 385 at the UAB	
1. Delay in wound healing	4 (1%)*
2 with vasculitic neuropathy	
2 with chronic inflammatory demyelinating polyneuropathy	
2. Post-traumatic neuralgia	2 (0.5%)

* All received steroid immediately after the biopsy.

and 3-0 nylon suture outside. An elastic bandage is applied locally to reduce the accumulation of blood and fluid. The patient may be up on the same day, but sitting with the leg in a dependent position for long periods or running is discouraged. Local pain is controlled with mild narcotics. The suture is removed in 7-10 days.

Following the sural nerve biopsy, invariably there is sensory loss over the lateral aspect of the foot corresponding to the sural nerve territory. Gradually this area decreases in size, but a quarter-sized area remains permanently insensitive to pin-prick.

There are two types of nerve biopsy: fascicular biopsy and whole biopsy. In fascicular biopsy, a few fascicles of nerve are biopsied, in order to lessen permanent sensory loss and long-term dysesthesia (Dyck and Lofgren 1968). However, studies have shown that there is no significant difference in the areas of sensory loss five or more years after sural nerve biopsy in fascicular biopsy compared with whole nerve biopsy (Pollock *et al.* 1983). Furthermore, fascicular biopsy may miss the vasculitic change in the perineurial space in cases of vasculitis because this is where splitting is made in the fascicular biopsy (Dyck *et al.* 1972). This is the most important reason against the fascicular biopsy, because vasculitis is one of the prime indications for the nerve biopsy. Therefore, I have concluded that there is no justification for fascicular biopsy. We routinely perform only the whole nerve biopsy.

Serious side-reaction following the sural nerve biopsy is rare. Significant pain or paresthesia was noted in 10% of patients one year after the biopsy (Stevens *et al.* 1975). Asbury and Connolly (1973) noted serious side-reaction in two cases out of 103: post-traumatic neuroma in one and pain in one. Among 385 sural nerve biopsies, we had post-traumatic neuralgia lasting one year in 2 cases (0.5%) and delayed wound healing in 4 cases (1%) (Table 6). In these latter four cases, steroid was administered for vasculitic neuropathy or chronic inflammatory neuropathy immediately after the biopsy, contributing to delayed wound healing. We have not observed any troublesome side-effects

in any of our cases two years after the biopsy. In Pollock's series (1983), there was no longterm pain or paresthesia in any of their cases five or more years after nerve biopsy.

PROCESSING OF THE BIOPSIED NERVE

Immediately after the biopsy, the nerve is gently

straightened, stretched, and placed on a silicone pad in dissecting dish for 15 minutes with pins at each end. This is important in reducing the contraction artifact. Asbury and Connolly (1973) stretch and apply the nerve to a thin strip of index card for 1 minute prior to being immersed in fixatives. Dyck and Lofgren (1968) suspend a biopsied nerve in the fixative with a tiny weight at the end.

The biopsied nerve is processed for the frozen sec-

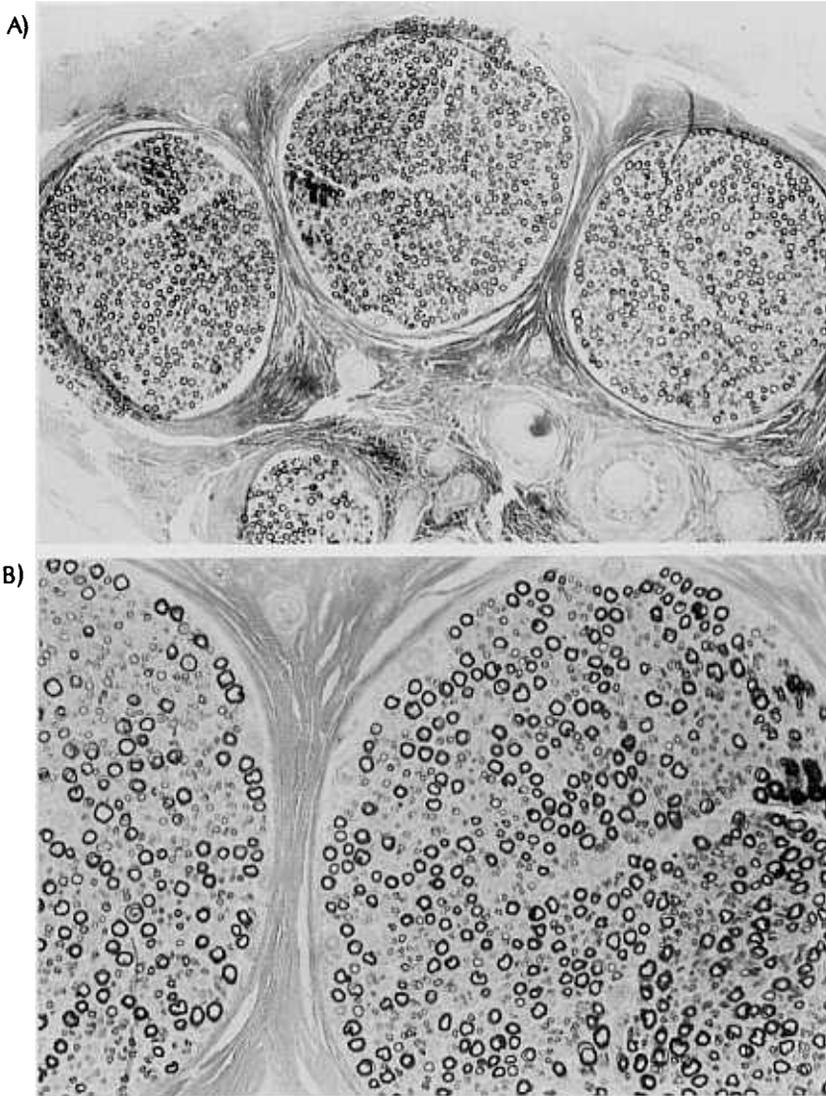
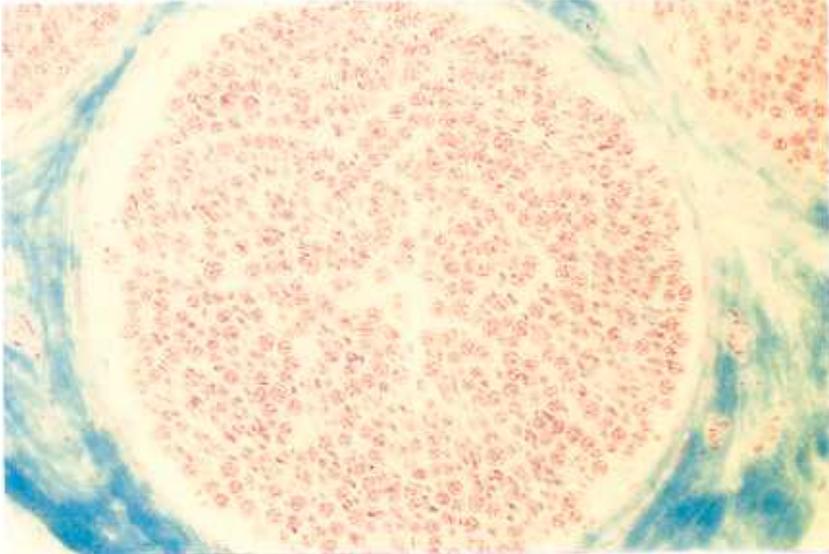


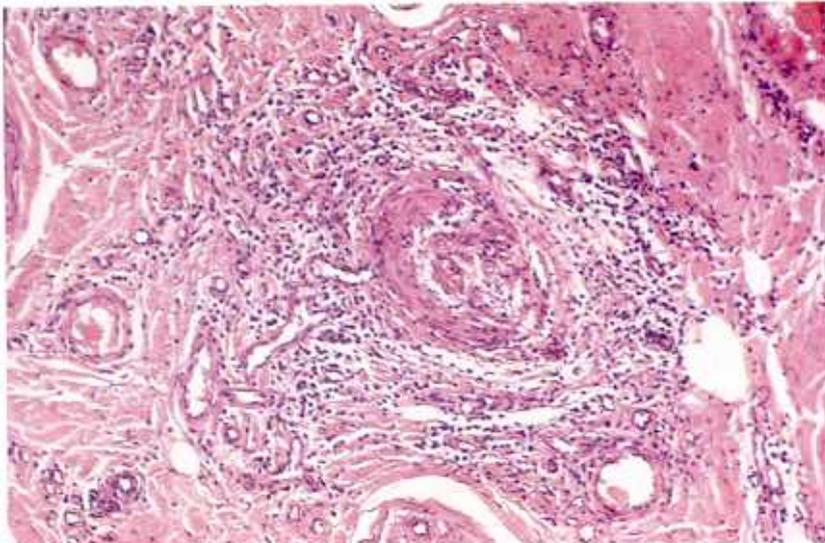
Fig. 3. Normal nerve. A) Four nerve fascicles represent half of the sural nerve biopsy specimen. Normally, each nerve fascicle is filled with myelinated fibers. Arterioles are noted in the perineurial space in the center of nerve. Paraffin section, Kultschistky's stain, $\times 100$. B) Normally, the various sized myelinated fibers fill the nerve fascicle. Ratio between the axon diameter and myelin thickness is 2:1 normally in large as well as small myelinated fibers. Kultschistky's stain, $\times 200$.

tion, paraffin section, semithin and EM section and teasing. We process all of our specimens for the frozen section first in order to make a fast and definite diagnosis. Rapid diagnosis is critical in cases of vasculitis since an immunosuppressive therapy should be instituted as soon as the diagnosis of vasculitis is made. In practice, the diagnosis of vasculitis can be made

within 15-30 minutes after the biopsy (Oh 1985). Metachromatic neuropathy can be diagnosed only with the frozen section since metachromatic granules are stained as such with cresyl-fast violet on frozen section alone. Other advantages of the frozen section are easy detection of myelin-digestion chambers and relative ease of preserving the longitudinal sections



Color Fig. 1. Normal nerve, transverse section. One nerve fascicle is filled with "red" myelinated fibers and surrounded by green perineurial connective tissue. Frozen section, Modified trichrome, $\times 200$.



Color Fig. 2. Active necrotizing vasculitis. Intramural infiltration of mononuclear inflammatory cells, fibrinoid necrosis of musculorum layer and near occlusion of vessel due to endothelial thickening are noted in an arteriole in the perineurial space. Paraffin section, H & E, $\times 400$.

straight. The latter is critical in recognizing segmental demyelination. These are all achieved in sections stained with modified trichrome (Harati and Matta 1979) and H & E with Surgipath Harris hematoxylin. Normal nerve on the frozen section stained with modified trichrome shows the nerve fascicles filled with myelinated fibers (Color Fig. 1).

The paraffin section is needed to identify amyloid by Congo-red staining and to delineate the detailed structures of cells and vessels. When semithin EM section is not available, the population of myelinated fibers, distribution of the nerve fiber according to the fiber diameter, and the relationship between the axon diameter and myelin diameter can be studied with paraffin section stained with Kulschistky's stain. With this stain, myelin is stained black (Fig. 3). The semithin EM section has been most commonly used for the peripheral nerve pathology in the recent years. This section is best to study in detail the axon-myelin relationship, to recognize onion-bulb formation and clustering of regenerated fibers, and to calculate the density of myelinated fibers. The semithin EM section is the only means of detecting thin-myelinated fibers (remyelination) with confidence. Teasing of nerve is best for documenting segmental demyelination, and can also recognize the nerve fibers with myelin ovoids (axonal degeneration). With teasing of nerve, one can study the relationship between the internode length and the fiber diameter. Teasing is mainly used as a research tool at this time because of the time-consuming nature of the technique. Electron microscopic study is known to be essential in diagnosis of certain diseases such as ataxia telangiectasia, Krabbe's disease, Fabry's disease, Tangier's disease, or neuroaxonal dystrophy by showing the distinct ultrastructural features (Dyck *et al.* 1984) and in studying unmyelinated fibers.

VASCULITIC NEUROPATHY

Vasculitis in the sural nerve biopsy is diagnostic of vasculitic neuropathy and vasculitis. Vasculitis is histologically characterized by the intramural infiltration of inflammatory cells and necrosis of vessel walls. Vasculitis is usually observed in small arteriols in perineurial or epineurial spaces.

Peripheral neuropathy is common in systemic vasculitides. Neuropathy is present in 60% of cases of polyarteritis nodosa and in 64% of cases of the Churg-Strauss syndrome (Frohnert and Sheps 1967; Chumbley *et al.* 1977). Vasculitis tends to involve medium and small sized arteries in many systemic vasculitides. Since the vasa nervorum in the peripheral

nerve fall directly into the spectrum of medium and small sized arteries, it is not surprising that peripheral neuropathy is a common manifestation of systemic vasculitides.

Peripheral neuropathy in systemic vasculitides is manifested in various forms: mononeuropathy, plexus neuropathy, mononeuropathy multiplex, asymmetrical polyneuropathy, and symmetrical polyneuropathy (Wees *et al.* 1981; Moore and Fauci 1981). Until recently, mononeuropathy multiplex has been regarded as the classical manifestation in vasculitis neuropathy. However, our study showed that symmetrical and asymmetrical polyneuropathy are also common (Wees *et al.* 1981).

Nerve conduction studies are vital to the work-up of patients with suspected systemic vasculitis for two reasons: (a) adequate nerve conduction tests can detect asymptomatic peripheral neuropathy, and (b) abnormal sural nerve conduction is a prerequisite to the demonstration of vasculitis on biopsy of this nerve (Wees *et al.* 1981).

As discussed above, whole nerve biopsy should be obtained in cases suspected of vasculitic neuropathy. The sural nerve biopsy should be done before any steroid treatment is initiated. It is necessary to cut multiple sections from different levels of the specimen since the vasculitis is multifocal and segmental. It has been our repeated experience that only a few sections of the biopsied nerve show the diagnostic change.

To render a definite diagnosis of vasculitic neuropathy, the unmistakable histological features of "vasculitis" must be present: active, inactive, or healed necrotizing changes and infiltration of inflammatory cells within the vessel wall (Fig. 4; Color Fig. 2). Perivascular infiltration of inflammatory mononuclear cells without intramural necrosis or cellular infiltration is an early and mild change in vasculitis (Dyck *et al.* 1972). This alone is not enough to be diagnostic of vasculitis because similar finding is observed in inflammatory neuropathies. However, there are some histological features which are helpful in differentiating these disorders: in vasculitic neuropathies, axonal degeneration is the predominant finding, while in inflammatory neuropathy, segmental demyelination and endoneurial inflammatory cells are typical findings. Thus, the diagnosis of probable vasculitis is made when perivascular infiltrations of inflammatory cells are present together with axonal degeneration if the clinical findings are compatible with vasculitis (Wees *et al.* 1981). Various patterns of degeneration of fibers are noted, ranging from central fascicular degeneration to selective nerve fascicular degeneration depen-

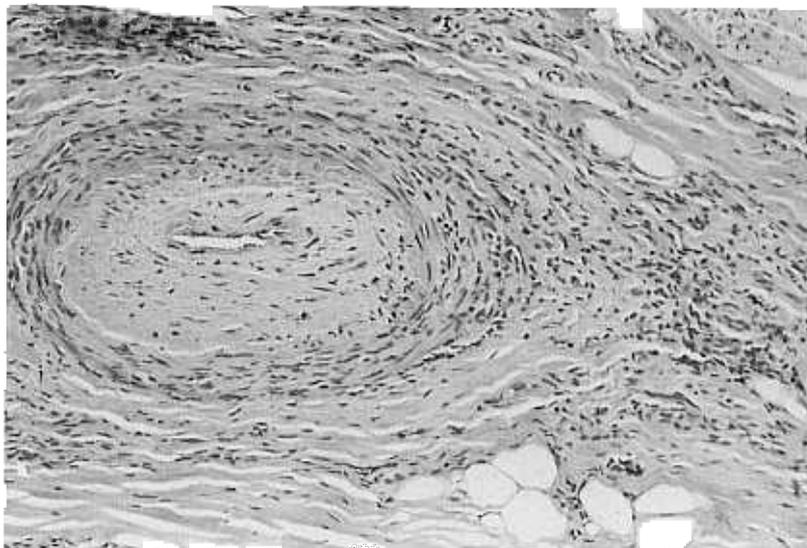


Fig. 4. Inactive vasculitis in the epineurial space in a case of vasculitic neuropathy. Near occlusion of arteriole due to endothelial thickening. A few mononuclear inflammatory cells are present in the muscular and adventitial layers of arterioles. This represents inactive or healed vasculitis. Paraffin section, H & E, $\times 200$.

ding upon the severity of neuropathy. The central fascicular atrophy is typical of ischemic neuropathy and is seen in vasculitic neuropathy (Dyck *et al.* 1972). Selective nerve fascicular degeneration has been observed predominantly in vascular neuropathy (Color Fig. 3) (Harati and Niakan 1986). It should be emphasized that any combination of these changes is found in a single sural nerve biopsy in cases of vasculitic neuropathy.

In recent years, paraneoplastic vasculitic neuropathy was reported (Johnson *et al.* 1979; Vincent *et al.* 1986; Slaughter *et al.* 1988). This entity is characterized by asymmetrical polyneuropathy, high sedimentation rate and high spinal fluid protein. Sural nerve biopsy is characterized by epineurial microvasculitis and axonal degeneration of the nerve fibers.

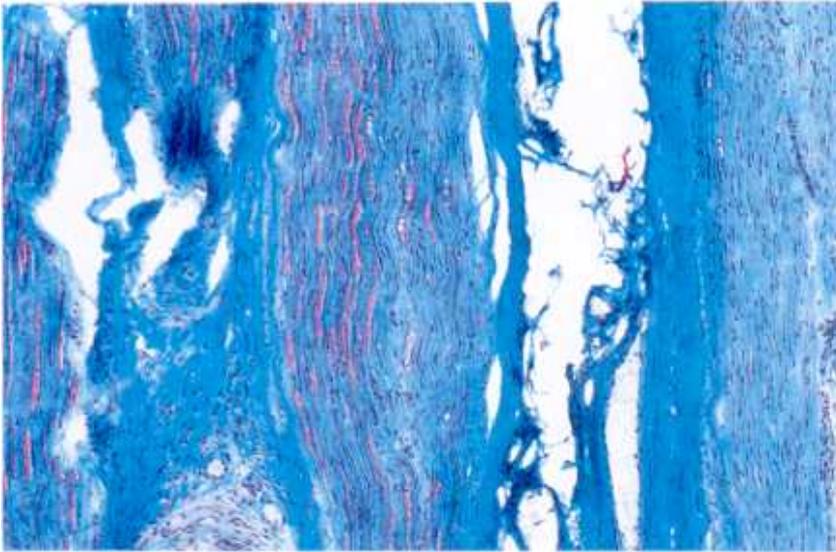
Vasculitic neuropathy was also reported in neuropathy associated with Lyme's disease caused by *Borrelia burgdorferi* spirochet (Vallat *et al.* 1987; Campovolo and Meier 1986). Nerve biopsy showed microvasculitis or perivascular inflammatory cells in the epineurial or perineurial space and axonal degeneration of the nerve fibers. In acquired immunodeficiency syndrome (AIDS), vasculitic neuropathy was reported in two cases of mononeuritis multiplex (Dalakas and Pezeschkpour 1988; Lange *et al.* 1988).

AMYLOID NEUROPATHY

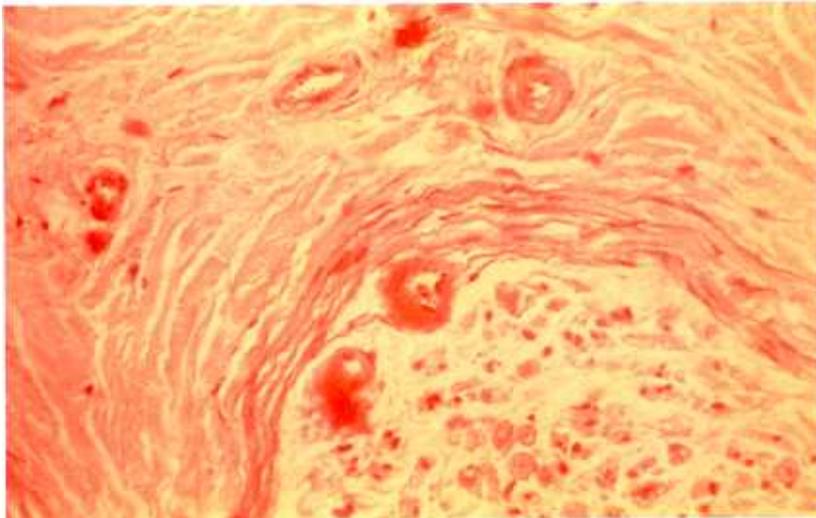
Amyloid deposit in the nerve biopsy is diagnostic of amyloid neuropathy and amyloidosis. Nerve biopsy is the diagnostic test of choice in any suspected cases of amyloid neuropathy.

Amyloid neuropathy is broadly divided into two major categories: hereditary and non-hereditary. Non-hereditary amyloid neuropathy includes a primary type associated with primary amyloidosis and a secondary type associated with malignant dysproteinemia, such as multiple myeloma, Waldenström's macroglobulinemia, or H-chain disease. Even though each type of amyloid neuropathy has distinct clinical features, they often share common characteristic features: sensory neuropathy, dysautonomia, and other organ involvement.

The hallmark of amyloid neuropathy is amyloid in the nerve. Amyloid is histochemically Congo-red positive and green birefringent after Congo-red with polarized light (Color Fig. 4). Thus, Congo-red staining of a biopsy specimen which is then examined by polarizing microscopy is the single best procedure for the diagnosis of amyloid (Cohen 1975). Using fresh-frozen sections, Trotter and Engel were able to demonstrate amyloids quickly and clearly using crystal-violet stain in biopsied muscles in ten cases of amyloid



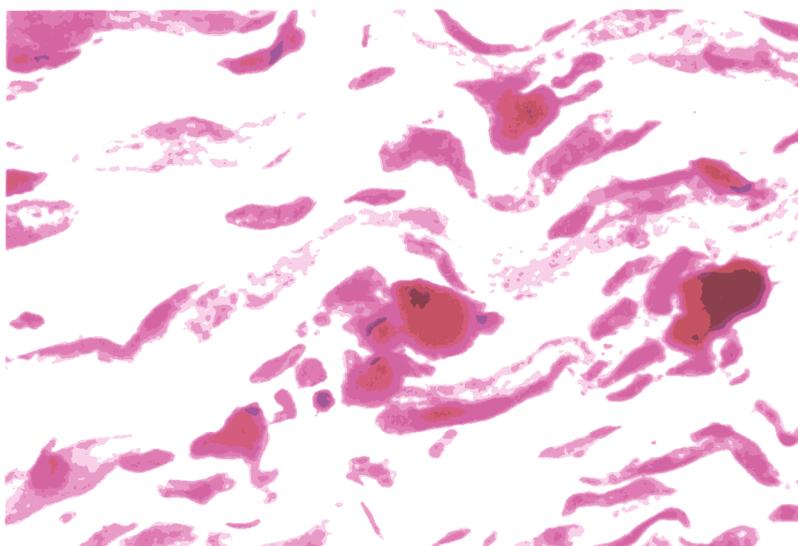
Color Fig. 3. Selective fascicular atrophy. One nerve fascicle contains many red myelinated fibers while other fascicles have none. This represents selective fascicular atrophy indicative of ischemic neuropathy. Occlusion of vasa nervorum supplying blood to one nerve fascicle proximally produces complete degeneration of nerve fibers distally. Frozen section, Modified trichrome, $\times 100$.



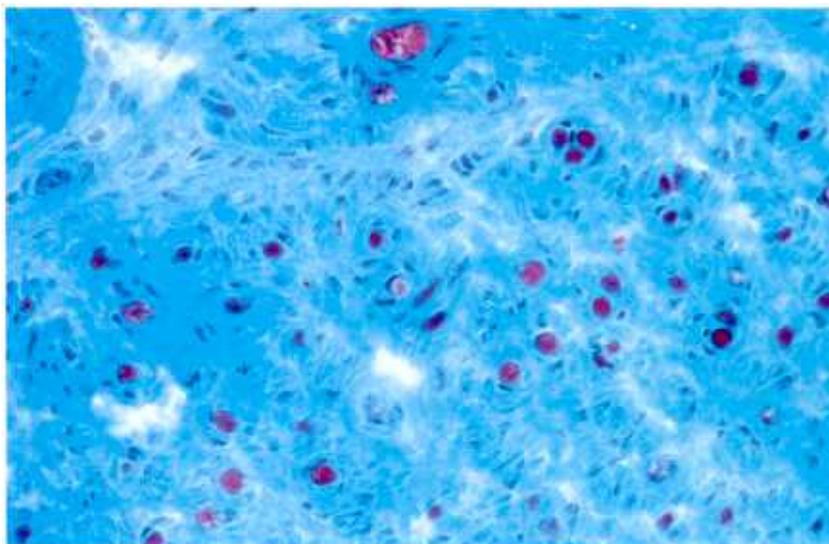
Color Fig. 4. Amyloid deposit. Homogenous red deposits in the wall of two endoneurial arterioles represent amyloid. Under polarized light, these deposits are reflected as apple green in color. Paraffin section, Congo-red stain, $\times 200$.

neuropathy while amyloid deposit was rarely observed in the biopsied nerves (1977). We use crystal-violet stain on frozen section to screen for amyloidosis but confirm or rule out amyloidosis on paraffin section by Congo-red stain in every biopsied nerve. Three patterns of amyloid deposition were found in the

peripheral nerve: (1) amyloid deposit in extraneural connective tissue, (2) widespread endoneurial amyloid deposit, and (3) amyloid deposit within the walls of vasa nervorum both in epineurial and endoneurial spaces. The predominant nerve degeneration in amyloid neuropathy is axonal degeneration, involv-



Color Fig. 5. Metachromatic granules. Purple granules represent “metachromatic granules”, as normal color of nerve fiber for this stain is pink. Frozen section, Cresyl-fast violet, ×400.



Color Fig. 6. Onion-bulb formation. Onion-bulb formations are clearly visible around every “red” myelinated fiber. Thin lines surrounding myelinated fibers represent proliferated Schwann cell processes. There is about 50% depopulation of myelinated fibers. Frozen section, Modified trichrome, ×200.

ing smaller diameter fibers.

METACHROMATIC NEUROPATHY

The presence of metachromatic granules in the nerve is diagnostic of metachromatic neuropathy and of metachromatic leukodystrophy.

Metachromatic leukodystrophy (MLD) is an autosomal recessive disorder characterized by the accumulation of galactosyl-3-sulfate (sulfatide) in the brain, kidney, gallbladder, and peripheral nerve. Four forms of MLD have been recognized: late infantile, juvenile, adult, and multiple sulfatase deficiency. The enzyme, arylsulfatase A is deficient in the first three

forms. Its assay in blood leucocytes and cultured skin fibroblasts is used as a standard diagnostic test.

Nerve biopsy constitutes a rapid and reliable procedure for the diagnosis of MLD when biochemical assay is not available. Metachromatic granules are demonstrable in all cases. For demonstration of metachromatic granules, the biopsied nerve should be stained on frozen sections since metachromasia is best demonstrable with acidified cresyl violet stain (Color Fig. 5) (Olsson and Sourander 1969).

Metachromatic granules are accumulated in the perinuclear cytoplasm of Schwann cells, within macrophages, and in the vicinity of endoneurial capillaries. These metachromatic granules are stained brown instead of purple or blue with cresyl violet or toluidine blue. They are also PAS positive and methylene blue positive. These metachromatic granules are demonstrated in all forms of MLD, including multiple sulfatase deficiency.

HYPERTROPHIC NEUROPATHY

The presence of onion-bulb formation is diagnostic of hypertrophic neuropathy. Thus, hypertrophic neuropathy represents a pathological diagnosis, observed in many clinical entities. Among these, the hypertrophic type of the Charcot-Marie-Tooth disease

(hereditary motor sensory neuropathy [HMSN] type I) is best known. In Roussy-Levy syndrome, Dejerine-Sottas disease (HMSN type III), congenital hypomyelinating neuropathy, and Refsum's disease (HMSN IV), onion-bulb formation is the most prominent finding in the biopsied nerve. In CIDP, onion-bulb formation is seen in 43-10% of cases (Oh 1978; Prineas and McLeod 1976). Onion-bulb formation is also observed in hypertrophic mononeuropathy, which is characterized by focal enlargement of a single peripheral nerve (Peckham *et al.* 1982). Hypertrophic mononeuropathy is different from generalized hypertrophic polyneuropathy by the following characteristics: (1) it is sporadic; (2) only one site is involved; (3) it can be adequately excised and does not recur; and (4) it lacks systemic extraneural manifestations (Peckham *et al.* 1982).

HMSN type I is the most common hereditary neuropathy. It is inherited as an autosomal dominant trait and is clinically characterized by pes cavus and marked atrophy of the feet and lower legs resulting in a characteristic appearance sometimes described as "stork-leg" or "inverted champagne bottle leg". This neuropathy is slowly progressive. Roussy-Levy syndrome has the clinical features of both the Charcot-Marie-Tooth disease and essential tremor. Because of this combination of clinical features, Dyck and Lambert (1968) classified this syndrome as Type I HMSN. The

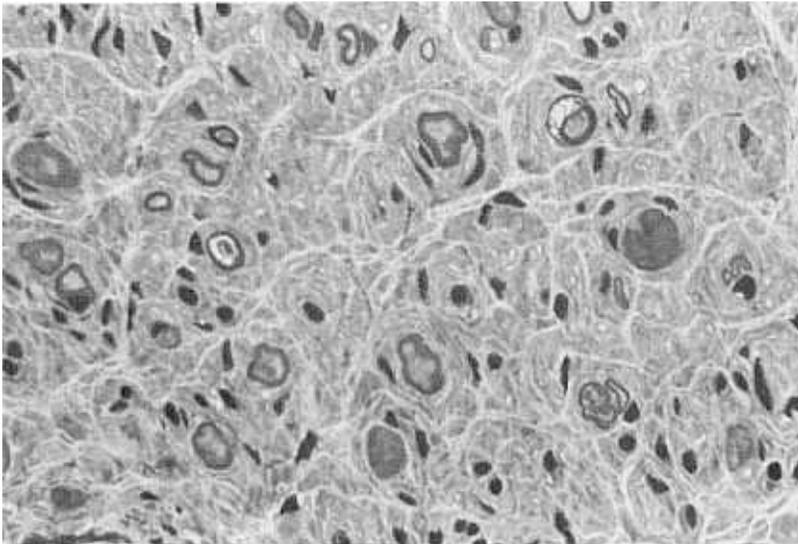


Fig. 5. Onion-bulb formation in a case of chronic inflammatory demyelinating polyneuropathy. Onion-bulb formations (arrows) are recognized by many fine lines (proliferated Schwann cell processes) surrounding nerve fiber. Some fibers with onion-bulb formations have more than one Schwann cells. Many fibers have thin myelinated fibers. Semithin sections, Toluidine blue, $\times 1000$.

Dejerine-Sottas disease, a recessively inherited disorder, usually begins during infancy or early childhood and is associated with slowly progressive weakness, thickened nerves, and a markedly increased cerebrospinal fluid protein. Congenital hypomyelinating neuropathy, most likely a variant of HMSN type III, has clinical features identical to those of the Dejerine-Sottas disease except for its onset at birth and absence of enlarged nerves. Refsum's disease (HMSN type IV: Heredopathia ataxica polyneuritiformis) is a hypertrophic neuropathy of autosomal recessive inheritance characterized by retinitis pigmentosa, ataxia, nerve deafness, ichthyosis, cardiomyopathy, and high CSF protein level. It is caused by an inborn error of metabolism in which an exogenous phytanic acid is accumulated in the tissue.

The pathological hallmark of hypertrophic neuropathy is onion-bulb formation (Fig. 5; Color Fig. 6 & 7). This term refers to the concentric laminated layers surrounding the nerve fiber as viewed in the transverse section. In electron microscopy, these laminated layers represent the intertwined and attenuated Schwann cell processes (Fig. 6) (Webster *et al.* 1967). Though onion-bulb formation is discernable

in the frozen section, it is best detected in the semithin section. When advanced, it is detectable even in the paraffin section. One way to identify onion-bulb formation in the paraffin section is to look for an increased number of Schwann cell nuclei. In advanced cases, onion-bulb formation is usually associated with prominent endoneurial and subperineurial spaces, decreased number of myelinated fibers and thin myelin.

Pathogenetically, onion-bulb formation is indicative of repeated demyelination and remyelination (Dyck 1969). Thus, hypertrophic neuropathy itself is indicative of demyelinating neuropathy.

INFLAMMATORY NEUROPATHY

The presence of inflammatory cell in the endoneurial space is diagnostic of inflammatory neuropathy, which is the type of neuropathy most commonly encountered in the practice of neurology. Inflammatory neuropathy is classified into two main categories: acute and chronic.

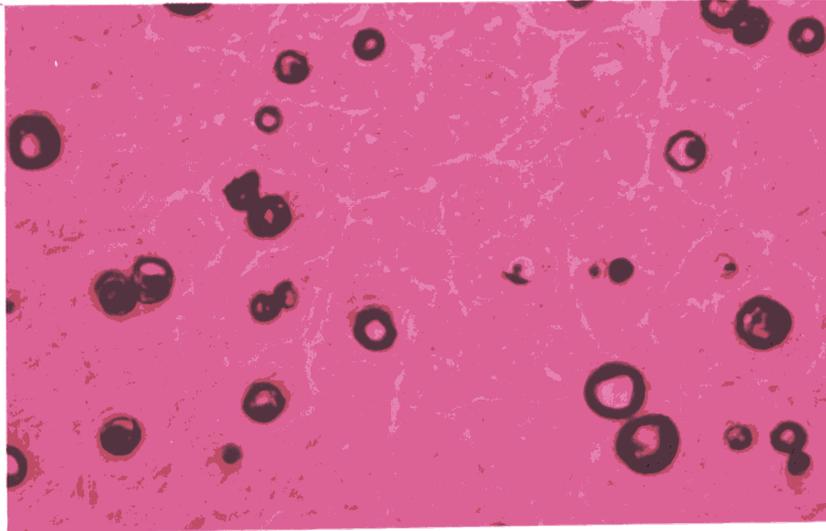
Acute inflammatory demyelinating neuropathy



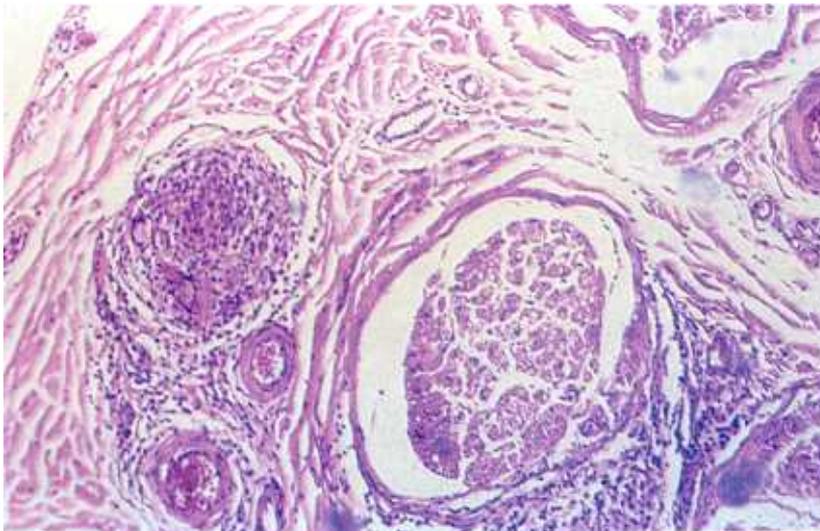
Fig. 6. Electron micrograph of onion-bulb formation in the sural nerve in a case of hypertrophic neuropathy in multiple sclerosis. Two Schwann cell nuclei and numerous processes surround myelin membrane with minimal alterations. Bar, 1 μm . (Published with permission of author and publisher. Ro Yi, Alexander B, Oh SJ: Multiple sclerosis and hypertrophic demyelinating peripheral neuropathy. *Muscle & Nerve* 6:312-316, 1983)

(AIDP), better known as the Guillain-Barre syndrome (GBS), is a well-known entity. GBS is characterized by acute ascending polyneuropathy. Progressive and usually symmetrical motor weakness, combined with hyporeflexia, is the cardinal clinical feature. CSF shows a high protein in most patients. Diagnosis of GBS is based on typical clinical features of acute progression

of diffuse polyneuropathy, high CSF protein, and nerve conduction abnormalities indicative of demyelinating neuropathy. In a majority of cases, diagnosis of GBS can usually be established without any difficulty. Thus, nerve biopsy is seldom indicated in the classical cases of GBS. However, we do recommend the nerve biopsy in atypical cases of GBS and in relapsing GBS. In relap-



Color Fig. 7. Onion-bulb formations. Onion-bulb formations are noted around all myelinated and demyelinated nerve fibers. Here they give an impression of small mounds with volcanic eruption. Paraffin section, Kultschisky's stain, $\times 400$.



Color Fig. 8. Sarcoid granulomata. Granulomatous vasculitis with two Langhans' giant cells, a few epithelioid cells, and many inflammatory cells in the wall of arterioles is clearly visible in the epineurial space. Many arterioles are surrounded by mononuclear inflammatory cells. One noncaseating granuloma located in the perineurial space outside of one nerve fascicle is partly visible. Paraffin section, H & E, $\times 200$.

ing GBS, our experience suggests that it is responsive to long-term steroid treatment. Thus, we do recommend the nerve biopsy before beginning steroid treatment for histological confirmation of this disorder.

In contrast to the ubiquitous presence of inflammatory cells in the peripheral nerve in the autopsy series in GBS, inflammatory cells are unfortunately not commonly observed in the nerve biopsies (Asbury *et al.* 1969). They were observed in 37% of the cases in our series and in 33% in Prineas's series (1972). The presence of inflammatory cells in the endoneurial space is the most specific finding indicative of inflammatory neuropathy (Fig. 7). Inflammatory cells are distinctly mononuclear, composed mainly of both small and large lymphocytes. Plasma cells are scattered among the lymphocytes. In some cases, perivascular infiltration of lymphocytes was seen only in the epineurial space. The most consistent finding in the sural nerve biopsy in GBS is segmental demyelination in our series and Prineas's study (1972). This is best observed in the teased fibers, the semithin plastic sections, and the longitudinal sections of the frozen section.

Chronic inflammatory demyelinating polyneuropathy (CIDP) has not been clearly delineated until recently and now is considered as a separate clinical entity on the basis of subacute progression of polyneuropathy, marked nerve conduction abnormalities, a high rate of relapse, and the response to

steroid treatment (Oh 1978; Prineas and McLeod 1976; Dyck *et al.* 1975; Dalakas and Engel 1981). In CIDP, there are two forms: monophasic form (subacute demyelinating neuropathy) and relapsing form (chronic relapsing neuropathy) (Oh 1978). The diagnosis of CIDP is based on the typical clinical features of subacute progression of polyneuropathy, high CSF protein, and marked nerve conduction abnormalities indicative of demyelinating neuropathy. We recommend the sural nerve biopsy in all patients with this disorder for the reasons described above.

The pathological hallmark of CIDP is primary demyelination, the most constant finding in the sural nerve biopsy (Fig. 8). Onion-bulb formation, a histological feature of repeated demyelination and remyelination, is the next most commonly observed feature in the sural nerve biopsy (Fig. 9). The presence of inflammatory cells, an expected feature in inflammatory neuropathy, is a rare occurrence. In Prineas's series, mononuclear cells were not observed in any of 23 cases (1976). We have observed mononuclear cells in two of 48 cases. In contrast, Dyck observed endoneurial inflammatory cells in 6 of 26 nerves and perivascular infiltration of cells in the peri- and epineurial space in 14 nerves (1975). When present, inflammation is not as prominent a feature as in GBS (Dyck and Arnason 1984). Usually, perivascular infiltration in the epineurial space is more common than endoneurial infiltration of cells.



Fig. 7. Endoneurial inflammatory cells in a case of the Guillain-Barre syndrome. Mononuclear inflammatory cells are scattered in the epineurial space. At the top, minimal perivascular collections of inflammatory cells are noted in the perineurial space. Paraffin section, H & E, $\times 200$.

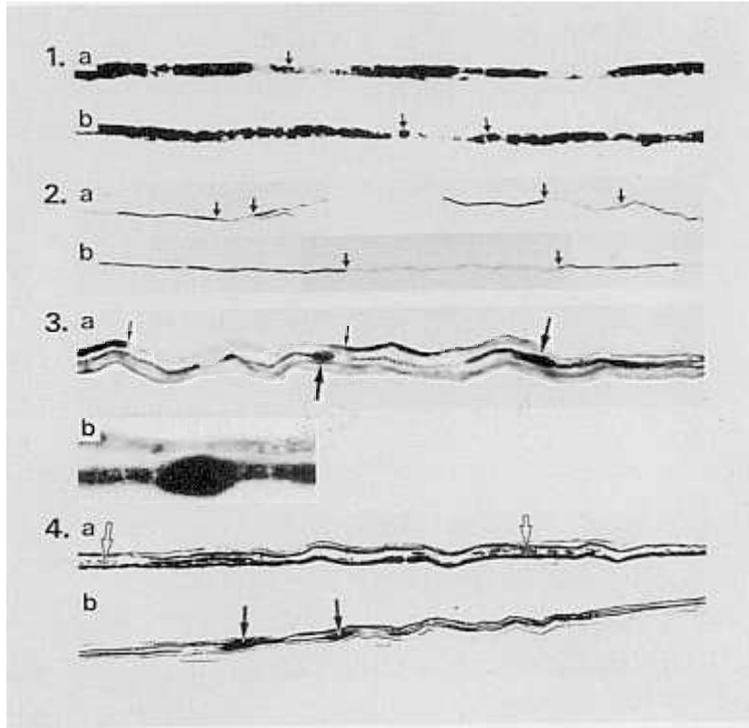


Fig. 8. Teased nerve fibers. 1: axonal degeneration: arrows indicate row of myelin ovoids. 2: demyelination: arrows indicate demyelinated segments. 3: tomaculous change. a) Thin arrows indicate a demyelinated segment. Thick arrows indicate "tomaculous change". b) Enlarged tomaculous change. 4: giant axons. a) White arrows indicate rows of myelin ovoids. b) Arrows indicate giant axons.

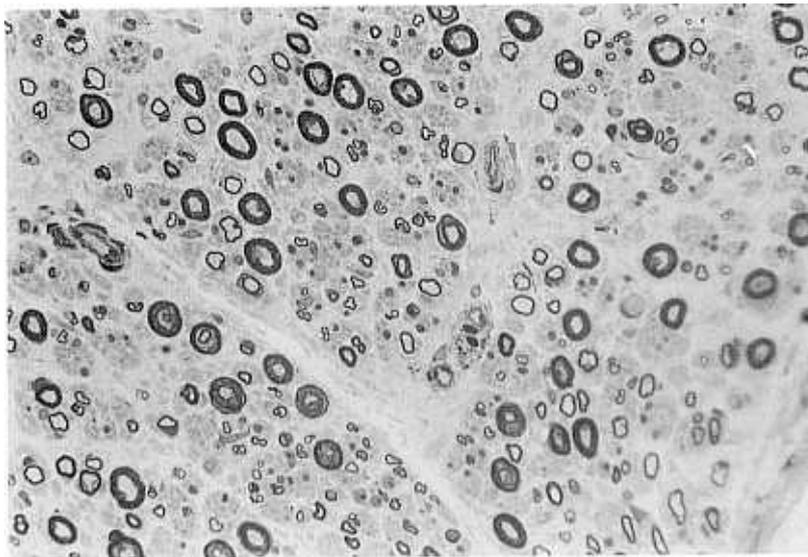


Fig. 9. Remyelination in a case of subacute demyelinating neuropathy. This myelin sheath in proportion to axon diameter (arrows) indicative of remyelination is seen in many fibers. Roughly the ratio between axon diameter and myelin thickness is 2:1. Many normally myelinated fibers are also seen. Semithin section, Toluidine blue, $\times 400$.

AIDP and CIDP were reported as the most frequently observed neuropathy in acquired immune deficiency syndrome (AIDS) due to HIV virus (Dalakas and Pezeschkpour 1988; Lipkin *et al.* 1985; Cornblath *et al.* 1987). CIDP was also reported in a single case of HTLV I myelopathy, another retroviral disease (Said *et al.* 1988). CIDP is well known to be a feature in many of dysproteinemic neuropathies associated with osteosclerotic myeloma, benign monoclonal gammopathy, and Waldenström's macroglobulinemia.

NONCASEATING GRANULOMATOUS NEUROPATHY

The presence of noncaseating granuloma in the nerve is diagnostic of sarcoid neuropathy and sarcoidosis, once leprosy has been ruled out by the AFB stain.

In sarcoidosis, microscopic granulomata were found in muscle in up to 60% of patients with active sarcoidosis while peripheral nerve involvement is less than 1% in sarcoidosis (Silverstein and Siltzbach 1969; Delany 1977). Thus, muscle biopsy is the procedure of choice for diagnosis of sarcoidosis if skin or lymph node biopsy is not diagnostic. Sarcoid neuropathy is characterized by a relapsing course, frequent facial neuropathy, and mononeuritis multiplex (Delany 1977). Rarely, symmetrical polyneuropathy was

reported.

In 1979, we have reported the first nerve-biopsy proven case of sarcoid neuropathy (Oh 1980). Since then, six cases of biopsy proved cases of sarcoid polyneuropathy were reported (Nemi *et al.* 1981; Gellaci *et al.* 1984; Vital *et al.* 1982).

Sarcoid granuloma is classically non-caseating granuloma consisting of epithelioid cells, Langhan's giant cells and lymphocytes (Fig. 10; Color Fig. 8). No organisms are found in sarcoid granuloma. Non-caseating granuloma have been observed primarily in the epi- and peri-neurial space (Oh 1980; Vital *et al.* 1982; Gellaci *et al.* 1984). Granuloma in the endoneurium was reported in only one case (Nemi *et al.* 1981). Granulomatous periangitis and panangitis were observed in the epi- and peri-neurial space in four cases (Oh 1980; Vital *et al.* 1982; Gellaci *et al.* 1984).

In practice, we recommend the combined muscle and nerve biopsy in patients clinically suspected of sarcoid neuropathy for two reasons: the diagnostic yield is high in muscle biopsy as described above; and granuloma was not always observed in biopsied nerves possibly because of the sampling error. In 3 of 4 cases with sarcoid neuropathy, the sural nerve biopsy did not show classical granuloma in our series.

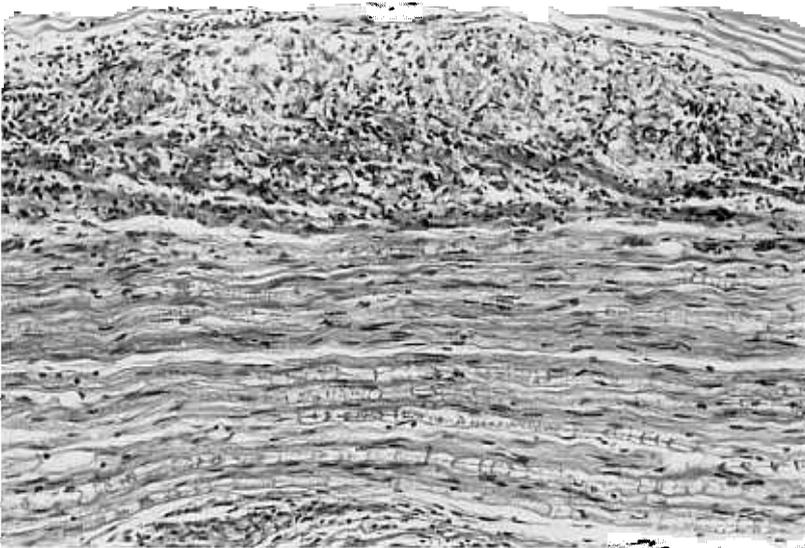


Fig. 10. Sarcoid granuloma in a case of sarcoid neuropathy. Noncaseating granuloma with many epithelioid cells and mononuclear inflammatory cells in the perineurial space in the upper half of figure. No granuloma or inflammatory cell is present in the nerve fascicle itself in the lower half. Paraffin section, H & E, $\times 200$.

CASEATING (NECROTIZING) GRANULOMATOUS NEUROPATHY

Necrotizing granulomatous neuropathy is diagnostic of neuropathy secondary to leprosy. Leprosy is an infectious disease caused by *Mycobacterium leprae* and characterized by its skin and peripheral nerve lesions. *Mycobacterium leprae* is the only bacterium which invades peripheral nerves in man and animals. It is classified into two polar types, tuberculoid and lepromatous, and a borderline (dimorphous; intermediate) type possessing some characteristics of each polar type. In addition, there is an indeterminate type which has not established itself into any of the three types.

The cardinal symptoms of leprosy are sensory loss caused by superficial neuropathy. Anesthetic depigmented skin lesions are an important finding. Another characteristic finding is thickened nerve. In tuberculoid leprosy, mononeuropathy multiplex is the typical clinical pattern, whereas asymmetrical or symmetrical polyneuropathy is most common in lepromatous leprosy.

The pathological features in nerve are different according to the types of leprosy (Weddell and Pearson 1975; Sabin and Swift 1975). In indeterminate leprosy, the nerve showed lymphocytic infiltration in the endoneurial and perineurial space. In tuberculoid leprosy, noncaseating or caseating granulomatous lesions are the most prominent feature. Granuloma is seen in the epi- and peri-neurial space as well as in the endoneurial space. Caseation may occur and produce large abscesses within the nerve. With healing, the nerve shows fibrosis and hyalization in the endoneurium and thick perineurial and epineurial sheathes. Bacilli are scanty and, when found, almost always in nerve. In lepromatous leprosy, the perineurial and endoneurial infiltration of macrophages and Schwann cells with AFB bacilli (foamy cells) and inflammatory cells is the cardinal feature. Massive bacilli are found in these foamy cells. In severe cases, the epineurium may be infiltrated by huge numbers of foamy cells, especially around blood vessels. With time there is endoneurial fibrosis. Intranuclear microabscesses may be present in either types, especially during an attack of erythema nodosum. In dimorphous leprosy, granuloma and endoneurial foamy cells are present. In all of these cases, the pathological diagnosis of leprosy should be made on the demonstration of acid-fast bacilli in the nerve by the Fite method (Fite *et al.* 1947).

In the majority of cases, diagnosis of leprosy is

usually made by observation of typical skin lesions and the presence of acid-fast bacilli from the skin smear or the skin biopsy. The nerve biopsy is imperative in diagnosis of primary neuritic leprosy in which neuropathy is the sole clinical manifestation without typical skin lesions or a positive skin smear. In these cases, the skin biopsy from anesthetic areas may fail to show histologic changes suggestive of leprosy (Jacob and Mathi 1988). In 77 patients with peripheral neuropathy without any known causes in a leprosy endemic area, Jacob and Mathi were able to confirm leprosy in 49.4% of cases by performing a nerve biopsy of the cutaneous nerve near the neurological deficit: nerve biopsy of the superficial radial sensory nerve in cases with "glove" anesthesia and of the superficial peroneal or sural nerve in cases with "stocking" anesthesia (1988). This study clearly shows the important diagnostic role of the biopsy of the cutaneous nerve in primary neuritic leprosy. The sural nerve biopsy was sensitive in diagnosis of leprosy in 89% of 18 patients with leprosy under treatment for varying periods (Haimanot *et al.* 1984). In one of two negative patients, skin biopsy showed tuberculoid leprosy and in another, skin biopsy showed no lesion. Additionally, the sural nerve biopsy may be helpful in identifying activity of leprosy in an apparently inactive case if a progressive neurological deficit occurs, since the study showed that positive sural nerve biopsy was found in 22% of 32 cases with such cases (Enna *et al.* 1970).

GIANT AXONAL NEUROPATHY

Giant axon in the nerve is diagnostic of giant axonal neuropathy and certain toxic neuropathies. Giant axonal neuropathy was first reported in 1972 by Berg *et al.* in a child with sensory neuropathy and curly hair. This neuropathy is seldom familial and is classically accompanied by curly hair (Jones *et al.* 1979). Central nervous system involvement has been reported in this neuropathy (Isisu *et al.* 1975; Mizuno *et al.* 1976).

Giant axons have been reported in certain toxic neuropathies: glue-sniffer's neuropathy, Huffer's neuropathy, and toxic neuropathy induced by n-hexane, methyl n-butyl ketone, acrylamide, and disulfiram (Korobkin *et al.* 1975; Oh and Kim 1976; Ansbacher *et al.* 1982; Allen *et al.* 1975; Rizzuto *et al.* 1977). N-hexane and methyl n-butyl ketone are widely used as solvents and as component of lacquers, glues, and glue- and lacquer-thinners. Huffer's neuropathy is peripheral neuropathy due to "huffing" of lacquer thinner. Thus, glue-sniffer's neuropathy and

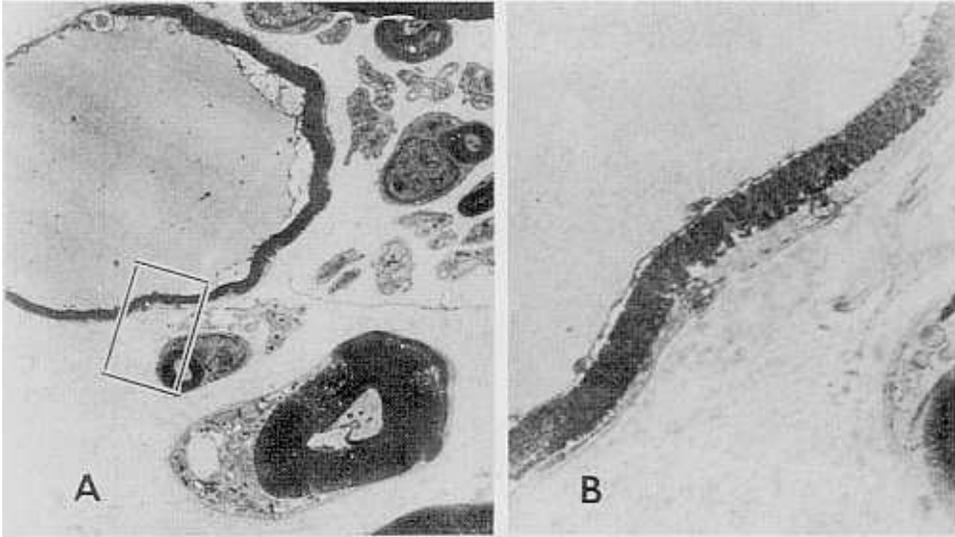


Fig. 11. Electron micrographs of sural nerve. *A*, Transverse section, showing “swollen axon” surrounded by thin or no myelin sheath ($\times 1800$). *B*, Axoplasm is filled with dense array of neurofilaments ($\times 9500$). (Published with permission from author and publisher. Oh SJ, Kim JM: Giant axonal swelling in “Huffer’s neuropathy”. *Arch Neurol* 33:583-586, 1976).

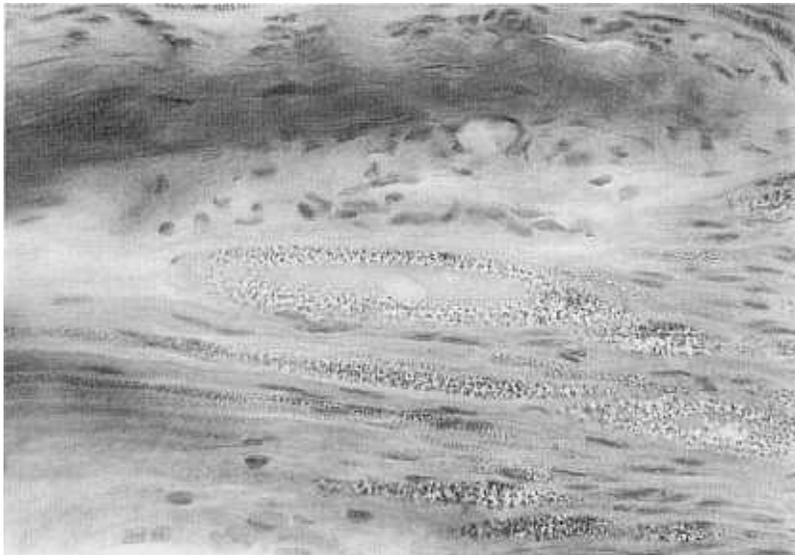
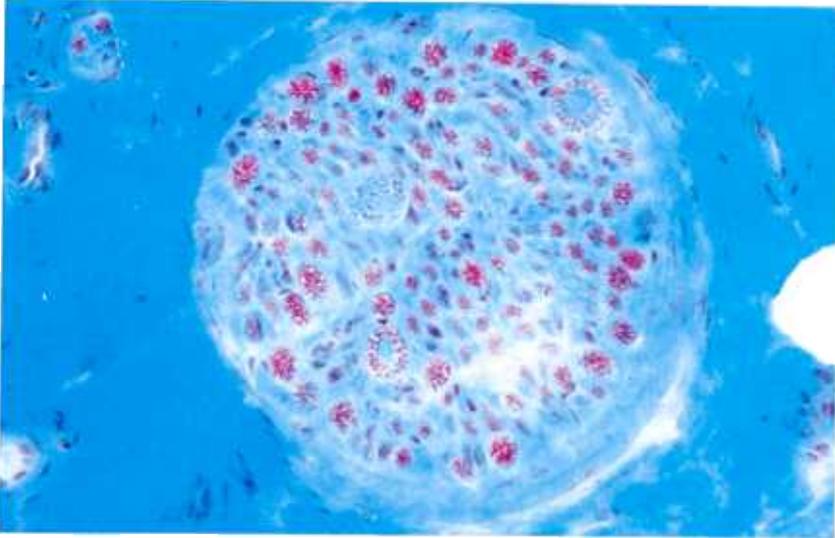


Fig. 12. Giant axon in a case of Huffer’s neuropathy. Giant axon (arrow) is surrounded by myelin sheath. Circle in the center of giant axon is bubble artifact. Frozen section, Modified trichrome, $\times 200$.

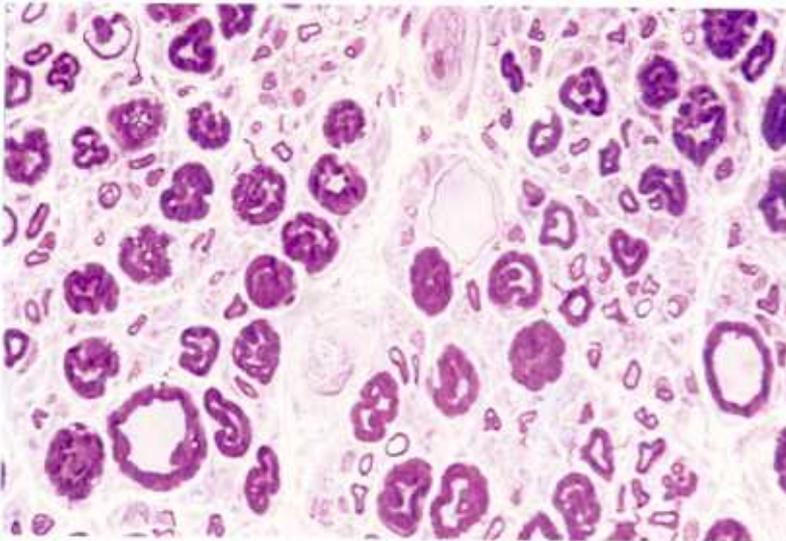
Huffer’s neuropathy are in essence due to inhalation of n-hexane or methyl n-butyl ketone. In disulfiram neuropathy, carbon disulfide, a metabolite of disulfiram, is responsible for giant axon.

Giant axonal swelling represents a focal mass of

neurofilaments surrounded by thin myelin (Fig. 11). Swelling ranged from 2 to 3 times the original diameter of the fibers and is usually associated with increased paranodal gap (Fig. 8). Giant axonal swelling is best seen in the transverse sections by the modified



Color Fig. 9. Giant axons in the transverse section. Three giant axons are visible in one nerve fascicle. Normally, axon is stained green as a dot in the middle of "red" myelinated fibers. Giant axon is easily identified here as "green center" surrounded by thin "red" myelin. Giant axons are three times larger as normal fibers in diameter. Population of myelinated fibers is also decreased. Frozen section, Modified trichrome, $\times 200$.



Color Fig. 10. Giant axon. Two giant axons are surrounded by thin myelin sheath while one giant axon (center) has no myelin sheath. Semithin section, Toluidine blue, $\times 400$.

trichrome stain on the frozen sections and semi-thin sections (Fig. 12; Color Fig. 9 & 10). Axonal degeneration is the predominant features in these neuropathies.

Giant axon in the nerve is diagnostic either of giant axonal neuropathy, or toxic neuropathies induced by n-hexane, methyl n-butyl ketone, methyl n-ethyl ketone,

acrylamide, and diflusaliramide.

TOMACULOUS NEUROPATHY

"Tomacula" (Latin, sausages) in the nerve biopsy are diagnostic of tomaculous neuropathy. In 1975,

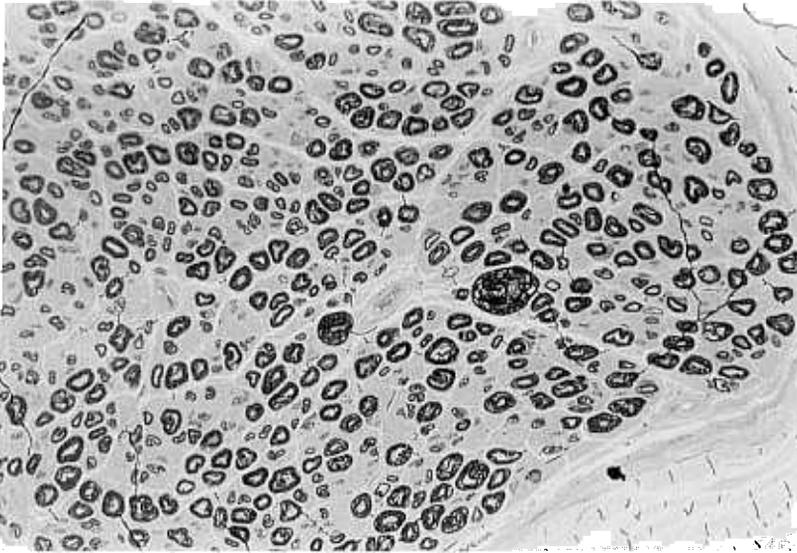


Fig. 13. Tomaculous formation in a case of tomaculous neuropathy. Two tomaculous formations with thick myelin layers are noted in the center. Diameter of larger tomaculous formation is twice that of the larger diameter normal fibers. Otherwise, relatively normal findings. Streaky lines are artifacts. Semithin section, Toluidine blue, $\times 200$.

Madrid and Bradley coined this term in 4 patients: 2 with recurrent familial brachial plexus neuropathy, 1 with a pressure-sensitive neuropathy, and 1 with a chronic distal sensorimotor neuropathy predominantly affecting arms.

Tomacula refer to the focal sausage-shaped swellings of myelin sheaths, best seen in the teased nerves (Fig. 8). However, this can be easily detected in the transverse and longitudinal sections on the frozen sections (Fig. 13; Color Fig. 11). There is no axonal swelling. Tomacula measured up to $27\mu\text{m}$ in diameter and from 80 to $250\mu\text{m}$ in length in Madrid's cases (1975). However, in our case tomacula ranged in diameter from 27 to $40\mu\text{m}$ in diameter (Joy and Oh 1989). Within the tomacula, the myelin sheath had an increased number of lamellae, two or three times the normal number of lamellae in the thickest myelin sheath of normal nerve (Behse *et al.* 1972).

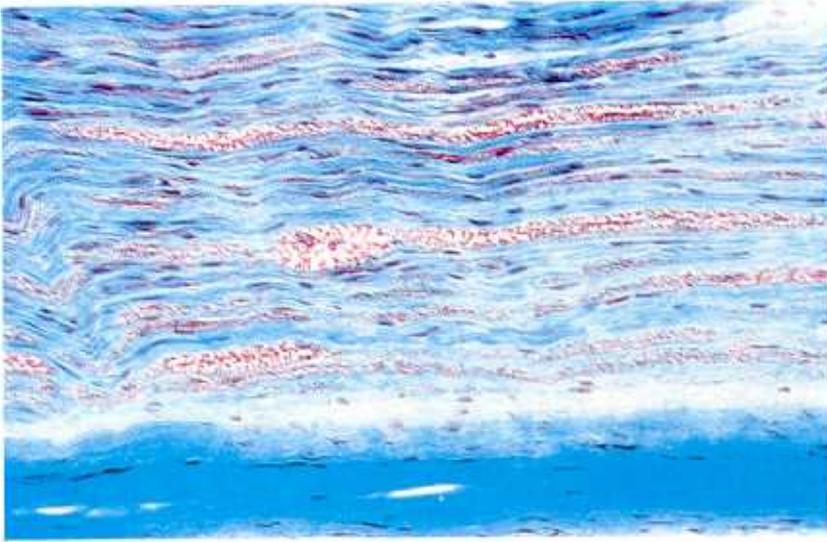
Tomaculous neuropathy was first described in Behse and coworker in 1972 in 5 patients with hereditary neuropathy with liability to pressure palsies. So far all of nerve biopsies from hereditary pressure neuropathy and recurrent familial mononeuropathy or brachial plexus neuropathy have shown tomaculous neuropathy (Earl *et al.* 1964; Fewings *et al.* 1985; Meier and Moll 1982; Pellissier *et al.* 1987). This neuropathy was also described in a case with brachial plexus neuropathy with Ehlers-Danlos syndrome (Schady and

Ochoa 1984), in a case with peripheral neuropathy with benign IgM monoclonal gammopathy (Vital *et al.* 1985), and in a case of chronic relapsing neuropathy (Joy and Oh 1989). Nerve conduction study in these patients usually shows features typical of demyelination. Segmental demyelination is the uniform finding in these cases. Onion-bulb formation is seen in some.

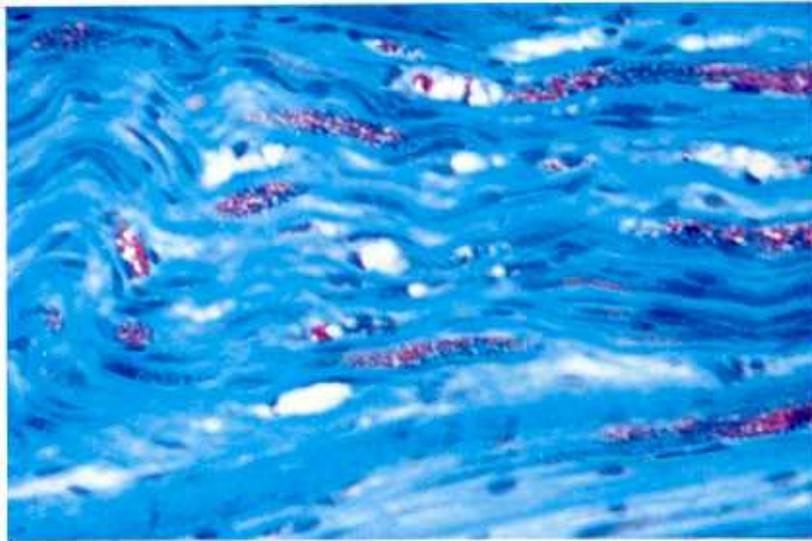
Tomaculous neuropathy represents demyelinating neuropathy and is most commonly seen in hereditary pressure palsy and familial recurrent mononeuropathy or brachial plexus neuropathy.

ISCHEMIC NEUROPATHY

Occlusion of the small arterioles and capillaries in the nerve is diagnostic of ischemic neuropathy, observed in diabetic neuropathy, vasculitic neuropathy, and arteriosclerotic neuropathy. Recent reports suggest ischemia as one possible factor in the pathogenesis of diabetic polyneuropathy (Dyck *et al.* 1986; Johnson *et al.* 1986). In contrast, ischemia does seem to be important in the pathogenesis of diabetic ophthalmoplegia and proximal asymmetrical diabetic neuropathy (Asbury *et al.* 1970; Dreyfus *et al.* 1957; Raff *et al.* 1968). In vasculitic neuropathies, occlusion of the arterioles may occur due to endothelial and intramural inflammation and proliferation as discussed



Color Fig. 11. Tomaculous formation: "Red sausage-like" myelin swelling represents tomaculous formation. Diameter is twice that of normal myelinated fibers here. Also seen are segmentally demyelinated fibers. Frozen section, Modified trichrome, $\times 200$.



Color Fig. 12. Myelin digestion chambers. Many empty chambers (myelin digestion chambers) are clearly seen along the pathway of myelinated fibers. Normal myelinated fibers are stained "red". Frozen section, Modified trichrome, $\times 200$.

above. Ischemia may be responsible for ischemic neuropathy due to severe arteriosclerosis (Eames and Lange 1967).

Small arterioles and capillaries in the perineurial and epineurial space shows occlusion due to the extensive fibrotic thickening and hyalization (Fig. 14). In

vasculitis, inflammatory cells may be present (Fig. 4). Because of the anatomical distribution of blood supply, ischemia produces degeneration of nerve fibers in a certain section of nerves, producing central fascicular atrophy (depopulation of fiber in the center of a fascicle) and fascicular atrophy (depopulation of fibers in

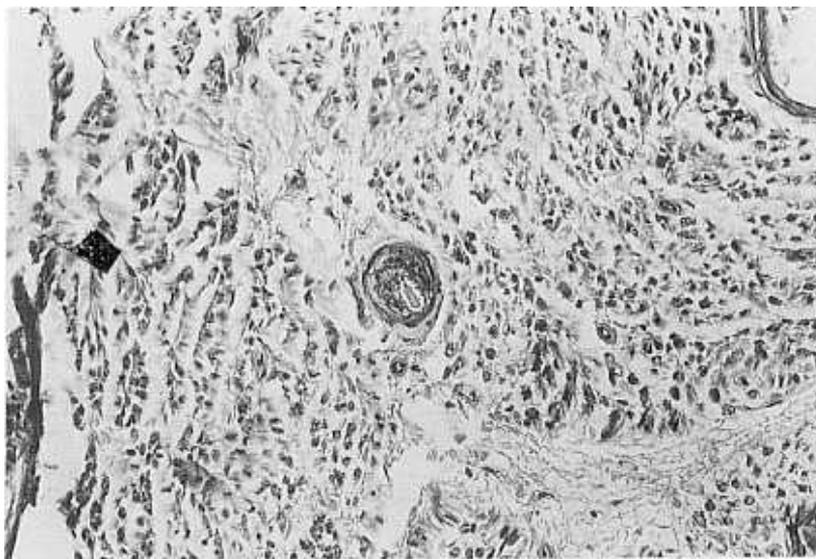


Fig. 14. Occlusion of the arteriole in a case of diabetic neuropathy. Near occlusion of an endoneurial arteriole due to arteriosclerotic endothelial thickening. Paraffin section, H & E, $\times 100$.

one or two fascicles). Thus, central fascicular atrophy and fascicular atrophy are used as the histological markers of ischemic neuropathy.

DEMYELINATING NEUROPATHY

Segmental demyelination in the nerve is diagnostic of demyelinating neuropathy. The classical example of demyelinating neuropathy is inflammatory neuropathy, either acute or chronic. In inflammatory neuropathy, inflammatory cells are often present in the nerve to make this diagnosis possible. Another example is hereditary hypertrophic neuropathy. However, there are many demyelinating neuropathies which are neither inflammatory nor hereditary. In these cases, segmental demyelination is the sole finding in the nerve without any histological clue for the exact etiology. Thus, etiology for neuropathy should be sought by other tests.

Segmental demyelination can best be observed in the teased nerves (Fig. 8). Demyelination can also be diagnosed by the thin myelin sheath in proportion to axon diameter in the semithin section, onion-bulb formation, or tomaculous change (Fig. 5, 6, 9 & 13; Color Fig. 6 & 7). In the longitudinal frozen section, segmental demyelination can be observed when the nerve is well stretched and the cut plane is uniformly flat.

Segmental demyelination in the nerve is diagnostic

of non-specific demyelinating neuropathy if other histological features diagnostic of specific neuropathies are lacking.

AXONAL NEUROPATHY

Axonal degeneration in the nerve is diagnostic of axonal neuropathy. Nutritional, alcoholic, vitamin deficiency, and most toxic neuropathies are the best examples. In these neuropathies, there is no histological feature indicative of a specific diagnosis, which should be made on the basis of other findings.

Axonal degeneration can best be diagnosed by the presence of myelin-digestion chambers in the frozen sections and by myelin ovoids in the teased nerves (Fig. 8; Color Fig. 12). Axonal degeneration is indirectly diagnosed by the presence of giant axon in the nerve. Axonal degeneration is also expressed by small clusters of small axons with thin myelin. This is most readily observed in the semithin transverse sections and represents repeated axon degeneration and regeneration (Schroeder 1968). In smoldering axonal degeneration, axon atrophy may be the sole finding indicative of axonal degeneration. Axon atrophy is best observed with electronmicroscopy by smaller axon diameter in proportion to normal myelin thickness.

Except for giant axonal and vasculitic neuropathies, most axonal neuropathies do not have any characteristic histologic features in the nerve indicative

of etiology. Thus, in these neuropathies, etiology should be sought by other tests as noted above.

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