

Invited Review

Pathological changes of human unmyelinated nerve fibers: a review

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Summary. In the cutaneous nerves, unmyelinated nerve fibers outnumber the myelinated ones but are scarcely analyzed, especially at autopsy. This indifference toward the pathology of unmyelinated nerve fibers may be due to the necessity of electron microscopic analyses and, more importantly, the obscurity of pathological alteration of unmyelinated nerve fibers in aging as well as in peripheral nerve disorders. The aim of this article is to review (1) the normal appearance including post-mortem changes, (2) the age-related changes, and (3) the pathological alteration in various neuropathic and non-neuropathic conditions, of unmyelinated nerve fibers in the sural nerve. For the complete analyses of sural nerve, qualitative and quantitative estimation of unmyelinated nerve fibers in all specimens should be recommended and it sometimes has an important diagnostic value.

Key words: Unmyelinated nerve fiber, Sural nerve, Collagen pocket, Schwann cell, Empty subunit

Introduction

Unmyelinated nerve fibers are one of the cardinal constituents of sural nerve. They are about three to five times more numerous than myelinated nerve fibers and are considered to represent postganglionic sympathetic efferents as well as sensory afferents subserving such modalities as pain and temperature. However, they have been occasionally neglected in routine pathological examinations, especially at autopsy. The relative indifference toward the pathology of unmyelinated nerve fibers could be well explained by this: (i) electron microscopic investigation is necessary due to their tiny size; (ii) because unmyelinated nerve fibers are unevenly distributed in the endoneurium, a morphometric analysis is required to evaluate the quantitative change of unmyelinated fibers (densities); (iii) other than the

changes in the density and the diameter histogram, pathological changes of unmyelinated nerve fibers are rather obscure; and (iv) the knowledge about the postmortem changes in unmyelinated nerve fibers are still scanty.

The aim of the present paper is to review the normal appearance, the age-related changes and the pathological alteration of unmyelinated nerve fibers in the sural nerve, the most frequently pathologically analyzed peripheral nerve and one of the most familiar structures for neurologists and neuropediatricians.

Normal morphology of unmyelinated nerve fibers in sural nerve

General morphology

The diameter of unmyelinated nerve fibers in the sural nerve ranges from 0.1 to 3.0 μm . Usually the diameter histogram shows a unimodal distribution, with the peak at 0.5-1.4 μm (Kanda et al., 1991b). The peak diameter varies among papers, presumably due to the difference in fixatives and postmortem durations. Hypertonic fixatives (e.g. Karnovsky solution) cause the shrinkage of unmyelinated axons, resulting in the shift of peak diameter toward the smaller size (usually less than 1.0 μm). Postmortem axonal changes include inflation of axons, and we found a significant relationship between the mode of frequency histogram of unmyelinated axon diameter and the time after death (Kanda et al., 1991b). Hence, if a subtle change like "axonal atrophy" of unmyelinated axons is required to be discussed, strict control of the content of fixatives and of the postmortem condition should be necessary.

Unmyelinated fibers are not evenly distributed in the endoneurial space: usually they tend to form a cluster composed of 10 to 30 unmyelinated axons (Fig. 1). This is the main reason why multiple, randomly-taken EM photos are necessary to evaluate the changes of unmyelinated nerve fibers quantitatively. Some of these clusters are situated between large myelinated nerve fibers, but many of them are in close proximity to the cluster of small myelinated nerve fibers. Sometimes,

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unmyelinated nerve fibers are difficult to distinguish from Schwann cell axoplasm. The following are morphological criteria for the recognition of unmyelinated axons: (1) the contour of unmyelinated axon is round or oval; (2) each axon is wrapped by Schwann cell process(es) and possesses apparent mesaxon; (3) the axoplasm of unmyelinated axons is almost similar to that of myelinated ones, except the relative abundance of microtubules/neurotubules; and (4) usually, the cytoplasm of the Schwann cells is more electron dense than the axoplasm (Fig. 2). However, in autopsy materials, the axoplasm sometimes shrinks and becomes as dense as Schwann cell cytoplasm (Fig. 3) (Kanda et al., 1991b). One Schwann cell does not wrap two or more myelinated nerve fibers; on the contrary, one to four unmyelinated axons are normally included in one Schwann cell subunit.

Collagen pockets

Longitudinally-oriented collagen fibers in the endoneurium are sometimes surrounded by Schwann cell process(es). These structures are called collagen pockets. Ordinarily they are not evenly distributed and are found to be concentrated in some areas in transverse sections

of the endoneurium. Because this structure usually possesses a mesaxon-like structure, some authors regard it as the result of the degeneration of unmyelinated axons, replaced by the collagen bundles. Thomas (1973) thought that they marked the original location of unmyelinated axons which have degenerated and disappeared. However, in their three-dimensional reconstructions of unmyelinated fibers, Carlsen et al. (1974) described that they were usually related to short isolated Schwann cell processes located externally within the Schwann cell subunits. Gamble (1964) regarded them as the consequence of active engulfing by Schwann cells of suitably-oriented elongated structures. We found a weak but significant correlation between the density of collagen pockets and that of the Schwann cell subunits without axons and did not observe any correlation between the densities of unmyelinated nerve fibers and collagen pockets. This suggests that the formation of collagen pockets is usually related to the proliferative activity of Schwann cells and does not mean the simple loss of unmyelinated axons (Kanda et al., 1991b). Thus, are the collagen pockets completely unrelated to loss of unmyelinated axons? Recently we reported a lot of clusters of collagen pockets in the cases of pure autonomic failure (PAF) (Kanda et al., 1998a).

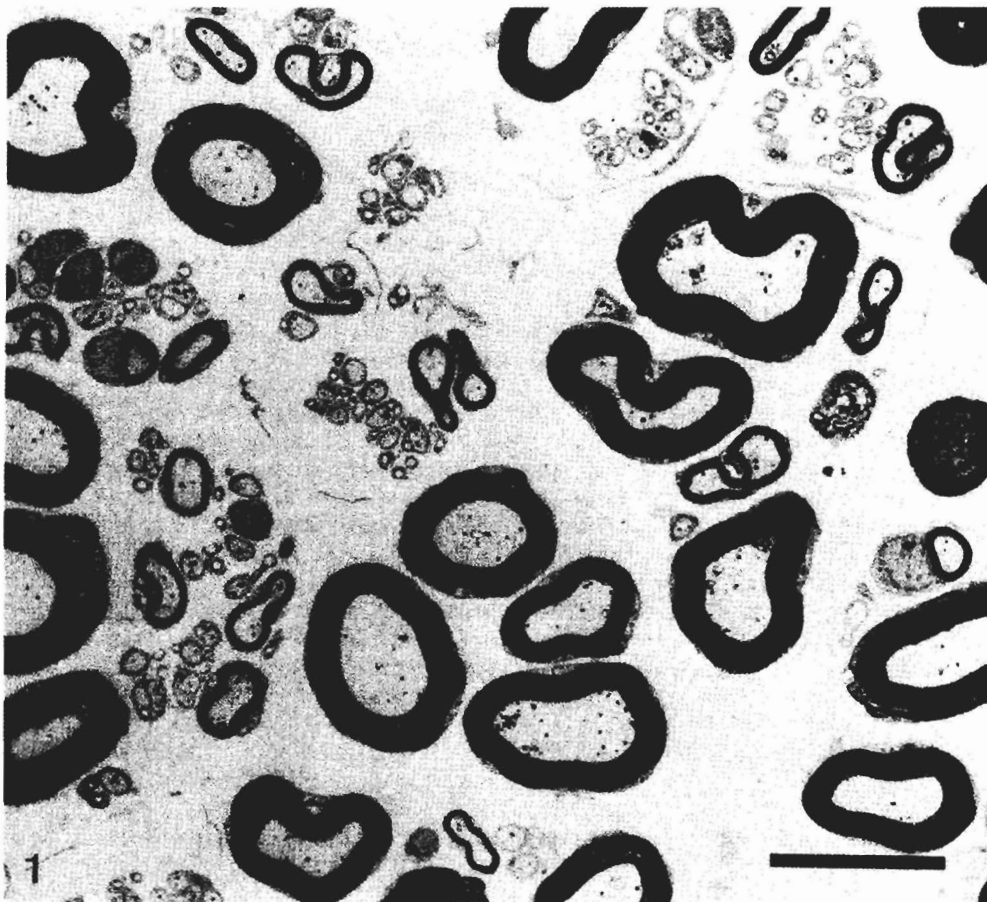


Fig. 1. Low power electron micrograph of biopsied sural nerve obtained from a patient of mild alcoholic neuropathy. Unmyelinated nerve fibers are not evenly distributed but scattered as small groups. Most of the groups of unmyelinated fibers are situated in proximity to small myelinated nerve fibers. Bar: 10 μ m.

Pathology of unmyelinated fibers

Because these collagen pocket clusters are rarely seen in normal subjects, we interpreted them as the reflection of recent, selective dropout of unmyelinated postganglionic sympathetic fibers in the sural nerve.

Quantitative analysis

Since quantitation of total unmyelinated axons in the

sural nerve is not practical in everyday pathological evaluation of biopsied sural nerve, unmyelinated nerve fiber density and histograms of the diameters of unmyelinated nerve fibers are routinely utilized for the quantitative analyses of unmyelinated axons in the sural nerve. We usually take EM photographs randomly, covering a total area of at least 0.03 mm² from more than three different fascicles. The final magnification

Table 1. Quantitation of human unmyelinated nerve fibers in the sural nerve.

AUTHORS	N	AGE (yr)	BIOPSY OR AUTOPSY	UNMYELINATED NERVE FIBER DENSITY (UMNFD; /mm ²)	AVERAGE UMNFD (/mm ²)	BIMODAL PEAK DISTRIBUTION IN ELDERLY SUBJECTS
Ochoa and Mair, 1969a,b	7	15-59	Biopsy	21,755-33,859	27,790	yes
Behse et al., 1975	6	17-47	Biopsy	19,000-56,900	34,130	no
Pollock et al., 1984	6	37-54	Biopsy	21,242-34,155	25,629	no
Jacobs and Love, 1985	24	0(-3w)-77	Autopsy	17,300-193,200		
	12*	21-77	Autopsy	17,300-41,600	27,380	yes
Kanda et al., 1991b	28	25-89	Autopsy	19,040-42,520	27,250	no

*: twelve cases aged 21 and over in the article by Jacobs and Love (1985) were re-selected and analyzed.



Fig. 2. High power electron micrograph of the same nerve as in Fig. 1. 1 and 2 indicate empty subunits, 3 a subunit containing one axon, 4 and 5 Schwann cell single protrusion (Kanda et al., 1991b). 1 and 5 possess a collagen pocket. Unmyelinated axons (A) are less electron dense and contain abundant microtubules compared to Schwann cell cytoplasm. Bar: 1 μ m.

used for measurement is $\times 10,000$. Randomly-taken photographs covering more than 0.02 mm^2 are generally accepted. The morphological criteria to separate unmyelinated axons from Schwann cell processes are described above; this is not necessarily an easy job, especially in severe, longstanding neuropathy specimens where the tiny axonal sprouts and reactive Schwann cell processes are intermingled.

We analyzed 28 sural nerves (age: 25-89 yrs) at autopsy and demonstrated that (i) unmyelinated nerve fiber densities ranged between 19,040 and $42,520/\text{mm}^2$; (ii) the diameter histogram for all of them showed a unimodal distribution, with the peak at $0.5\text{-}1.4 \mu\text{m}$; and (iii) no correlation could be found between the density of unmyelinated axons and the patient's age (Kanda et al., 1991b). This is the most expansive and detailed study for the unmyelinated nerve fibers of sural nerves in human adults so far. Several authors (Behse et al., 1975; Jacobs and Love, 1985; Kanda et al., 1991b; Ochoa and Mair, 1969a,b; Pollock et al., 1984) reported control value of unmyelinated nerve fibers as described in Table 1. The normal range of unmyelinated nerve fiber density varies among authors and the upper limit of the normal range sometimes exceeds twice the lower limit; however, it is astonishing that the average values of unmyelinated

nerve fiber density are close in most of the articles as far as adult materials are concerned. To sum up these reports, it would be reasonable to regard (i) bimodal distribution or (ii) density less than $15,000/\text{mm}^2$ as abnormal in adults, although there is still controversy as to whether a bimodal pattern in old subjects can be considered as an abnormal finding or not.

Since the quantitation of unmyelinated nerve fiber densities using EM is time-consuming and costly as described above, this technique is not routinely applied for all the biopsy specimens. Light microscopic evaluation, if possible, has the advantages that (i) it can cover wider areas than EM quantitation; and (2) it does not take so much time and can be performed at any laboratories. Johnson et al. (1994) reported a method to quantitate unmyelinated nerve fibers on glutaraldehyde-fixed, paraffin-embedded sections using anti-PGP 9.5 antibody. Even in well-made semithin transverse sections stained with toluidine blue, we can recognize unmyelinated axons as small, round or oval structures not well-stained as compared with surrounding, blueish Schwann cell cytoplasm. These non-EM evaluations require some knowledges on the EM appearances of unmyelinated axons to recognize unmyelinated axons in light microscopic specimen and the evaluation of fiber

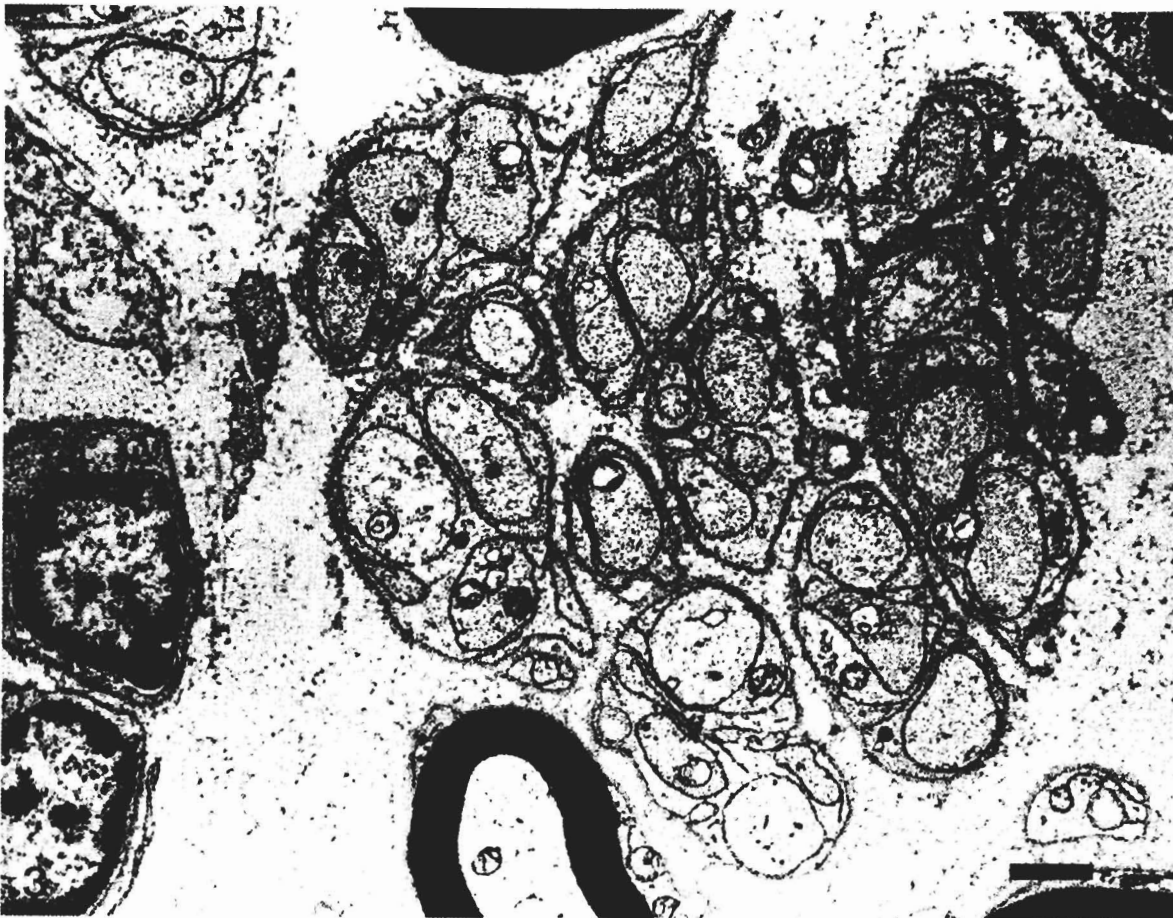


Fig. 3. Sural nerve autopsy specimen obtained from a patient (50F) of multiple sclerosis. The difference in electron density between axoplasm and Schwann cell cytoplasm has almost disappeared. Mesaxons are still recognizable but microtubules are not observed. Bar: $1 \mu\text{m}$.

size distribution is extremely difficult with non-EM evaluations. Because the initial change of unmyelinated nerve fibers is not the decrease of unmyelinated nerve fiber density (Kanda et al., 1991b), the application of these non-EM techniques is considered to be limited so far.

Functional components of unmyelinated nerve fibers in sural nerve

One of the major problems in the clinicopathological evaluation of the morphological changes of unmyelinated nerve fibers is the proportion of the two components (somatic afferent and sympathetic postganglionic efferent) in human sural nerve; this is still a subject of debate. Despite the fact that various efforts have been made to distinguish these two components histochemically (Matsubayashi et al., 1986; Hayashi et al., 1991; Kusunoki et al., 1991), there is no definite method to separate them completely. Hence, the exact percentage of these two modalities in unmyelinated fibers of human sural nerve is unknown so far. In the rat, Chad et al. (1983) showed that up to a quarter of the total population of peripheral nerve unmyelinated axons are sympathetic ganglia derived.

Extensive scrutinies of the sural nerve obtained from patients with selective involvement of postganglionic sympathetic nervous system may solve this problem. Chad et al. (1981) found a normal density and diameter histogram for unmyelinated axons of biopsied sural nerve from a patient after lumbar sympathectomy, and concluded that the sympathetic nervous system contributed few axons to the total population of unmyelinated axons in the sural nerve. Their result might be due to the wide range of normal unmyelinated nerve fiber density, obscuring the disappearance of a substantial number of unmyelinated nerve fibers. The mean unmyelinated nerve fiber density in 7 PAF patients was 40% less than in age-matched controls (Kanda et al., 1998a). Because sympathetic nervous system is believed to be selectively involved in PAF, this figure is suggestive of the exact percentage of sympathetic efferents in the unmyelinated nerve fibers of sural nerve.

Whether the various functional types of unmyelinated fibers (slow pain afferents, warmth afferents, sympathetic efferents) in the sural nerve are intermingled or segregated in exclusive fiber bundles also remains to be established (Thomas et al., 1993). Because fibers carrying specific modalities are known to run in discrete groups in some portions of the peripheral nervous system (Ravits et al., 1979; Thomas et al., 1993), it is possible that unmyelinated sympathetic efferents and unmyelinated sensory afferents are not evenly mixed, at least at the level of the sural nerve trunk. I found increased numbers of clusters of collagen pockets in PAF cases; this appear to reflect recent dropout of a group of sympathetic afferents and suggests grouping of unmyelinated fibers by modality at the level of the sural nerve trunk (Kanda et al., 1998a).

Age-dependent changes of unmyelinated nerve fibers

Unmyelinated nerve fiber density and diameter histogram of unmyelinated axons

Density of myelinated nerve fibers, especially that of larger ones, is known to decrease with age. As for the unmyelinated nerve fibers, quantitative data for the normal subjects are still scanty. Jacobs and Love (1985) studied 27 sural nerves from 'normal' subjects obtained at autopsy and described a consistent reduction in the density of unmyelinated nerve fibers with age. On the contrary, we did not recognize any correlation between the density of unmyelinated axons and the subject's age (Kanda et al., 1991b). Several papers using a smaller number of subjects also supported our results (Ochoa and Mair, 1969b; Behse et al., 1975). The discrepancies between our data and those of Jacobs and Love concerning the density of unmyelinated axons may be partly explained by the difference in the subject's age. In their paper, a marked decrease in the density of unmyelinated axons with age occurred mainly during the early years of life and the age-dependent reduction was not as apparent in the adult. The diameter histogram for all our 28 cases showed a unimodal distribution, regardless of age (Kanda et al., 1991b). Some authors described an increase in small axons in the elderly (Ochoa and Mair, 1969b; Jacobs and Love, 1985); this may be partly due to the differences in the criteria used to distinguish the tiny axons from the small Schwann cell processes.

Empty Schwann cell subunits

A conglomerate of two or more Schwann cell processes without unmyelinated axons, which is enclosed by a continuous basal lamina, is designated as an empty Schwann cell 'subunit' (Sharma and Thomas, 1975). Ochoa and Mair (1969b) reported that the density of empty Schwann cell subunits was about 1,245-3,700/mm² in men aged 34 and 59 yrs, respectively, while they were less than 1,000/mm² in individuals aged 32 and under. Schröder and Gibbels (1977) studied 2 senile control subjects (aged 83 and 88 yrs) and stated that the empty Schwann cell subunits might be related to aging or another cause of slow axonal wastage with Schwann cell proliferation. We found a significant correlation ($p < 0.01$; $r = 0.7098$) between the density of empty Schwann cell subunits and the subject's age (Kanda et al., 1991b).

The origin of this structure is still in dispute. Some authors thought of them as the residue of axonal loss (Ochoa and Mair, 1969b; Ochoa, 1970); others considered that they were pathological new additions of collateral Schwann cell appendages (Behse et al., 1975). Our quantitative study revealed that increases in empty subunits with age did not occur concomitantly with a decrease in unmyelinated nerve fiber density but

accompanied an increase in the number of Schwann cell profiles in a subunit and in the density of isolated Schwann cell projections. Because the density of Schwann cell nuclei related to unmyelinated nerve fibers did not increase significantly with age, the empty subunits are considered to be derived from an enhanced proliferative activity of Schwann cells without multiplication, rather than from the simple loss of unmyelinated axons (Kanda et al., 1991b).

Collagen pockets

Ochoa (1971) found no collagen pockets in fetal nerve before 18 weeks, and many authors believe that they are related to aging (Thomas and Ochoa, 1984). We found that the density of collagen pockets did not exceed 10,000/mm² in younger individuals and that higher values (more than 10,000/mm²) were observed in some of the older controls (over 50 yrs; seven out of 20 subjects) (Kanda et al., 1991b). However, because the value of collagen pocket density is so scattered among subjects, I consider this parameter is not superior to the other indices, including the density of empty subunits, as a sign of aging.

Other parameters

We demonstrated that the mean number of Schwann cell profiles per axon in Schwann cell subunits with axon showed positive correlation with age, and that the percentage of subunits containing unmyelinated axons (number of Schwann cell subunits with axon / number of all subunits x 100) revealed negative correlation with age (Kanda et al., 1991b). In terms of relative sensitivity for the detection of early and subtle changes in unmyelinated nerve fibers, we consider that these two indices are superior to the conventional assessment of unmyelinated axon density and diameter distribution. In

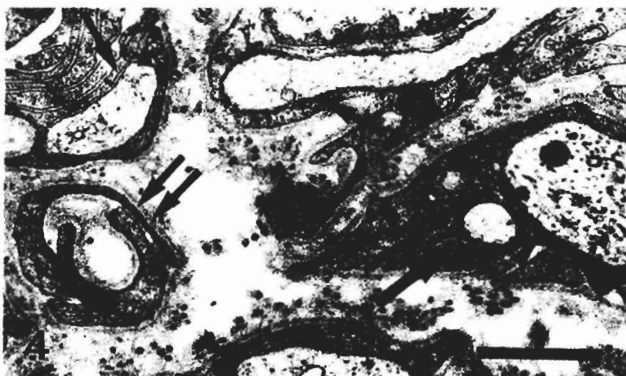


Fig. 4. Acute axonal changes of unmyelinated nerve fibers in acute sensory and autonomic neuropathy (41M). Some unmyelinated axons still possess abundant organelles in the axoplasm (arrowheads); however, the microtubules are obscured in most of them (arrows) and occasionally the whole axon has disappeared (two arrows). The latter structure is different from collagen pocket since no collagen fibers are included. Bar: 1 μ m.

addition, these two are not influenced by postmortem swelling of the axons and Schwann cells (Kanda et al., 1991b).

Pathological changes of unmyelinated nerve fibers in various neuropathies

Acute changes

Although the chronological morphological changes of myelinated fibers in acute nerve transection have been well described (Dyck et al., 1993), the sequence of wallerian degeneration of unmyelinated nerve fibers has not been studied as extensively as that of myelinated ones. Thomas and King (1974a) showed the ultrastructural changes of rabbit vagus nerve following transection. They described that the initial change, a loss of the microtubules, was apparent within 24 hrs. A proportion of axons was greatly enlarged and contained a profusion of organelles at 24-48 hrs after operation, and all the axons had degenerated after 7 days (Fig. 4). Although freely lying granular debris and material derived from degenerate organelles remained at this stage, most of it had disappeared by 15 days after nerve section. Only the flattened Schwann cell processes remained thereafter. This rapid process is the main reason why we do not encounter the acute changes of unmyelinated nerve fibers as frequently as those of myelinated fibers in sural nerve biopsy specimens.

Chronic changes

Usually, the initial pathological changes in most cases of peripheral neuropathy are the decrease in the density of myelinated nerve fibers, especially that of large myelinated fibers. In contrast, because unmyelinated nerve fibers possess the strong ability to sprout after injury, the earliest changes of unmyelinated nerve fibers in neuropathic condition include the increase in small unmyelinated axons. Since these small fibers sometimes do not have a complete mesaxon, it is not always easy to distinguish them from Schwann cell processes. Hence, a very initial change of unmyelinated nerve fibers may easily be overlooked if only the conventional unmyelinated nerve fiber density and fiber diameter distribution are utilized for morphological evaluation. In addition, the 'normal' range of unmyelinated nerve fibers is very wide; a neuropathic patient who has lost almost half of the unmyelinated nerve fibers may be interpreted to have 'normal' unmyelinated nerve fiber population, if his original unmyelinated nerve fiber density had been around 40,000/mm².

Behse (1990) suggested that the best indicator of fiber loss was an increase in the number of empty subunits. Behse and Buchthal (1977) reported that 6 out of 13 biopsies from alcoholic neuropathy patients showed decreased unmyelinated nerve fiber density and 4 presented increased number of empty subunits without

apparent loss of unmyelinated nerve fibers. We also found that the empty subunits were increased without any evidence of decrease in unmyelinated nerve fiber density by aging (Kanda et al., 1991b). In conclusion, for the evaluation of minimum and early changes in unmyelinated nerve fibers, a precise morphological estimation of Schwann cells associated with unmyelinated axons is as important as that of unmyelinated nerve fibers themselves.

Peripheral neuropathy with prominent changes in unmyelinated nerve fibers

(1) Acute pandysautonomic neuropathy

Acute pandysautonomic neuropathy is an uncommon disorder characterized by severe postganglionic sympathetic and parasympathetic dysfunction, with relative or complete sparing of somatic function. The pathology of sural nerve biopsy was characterized by the decrease in small myelinated nerve fibers and unmyelinated nerve fibers. The relative increase in empty subunits and collagen pockets was also described (Appenzeller and Kornfeld, 1973; Low et al., 1983; Feldman et al., 1991). Although Feldman et al. (1991) speculated that the diminished population of unmyelinated nerve fibers primarily represented a loss of sympathetic C fibers in this disorder, it should be reasonable to consider that sympathetic postganglionic as well as somatic afferents are affected in acute pandysautonomia and that the decreased population of unmyelinated nerve fibers are not necessarily with sympathetic modality.

(2) Diabetic neuropathy

Decrease in unmyelinated nerve fibers is common in diabetic neuropathy (Behse et al., 1977). Especially in "small fiber diabetic neuropathy (Said et al., 1983)" patients, who showed predominant loss of pain and

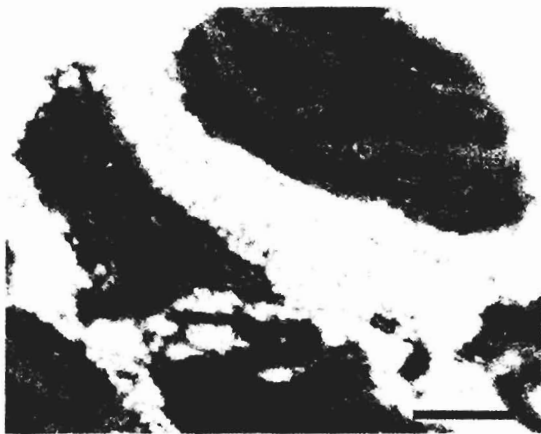


Fig. 5. Diabetic neuropathy with dysautonomia (59M). Most of the unmyelinated axons have disappeared and numerous empty subunits are seen. Also note the thickened basement membrane surrounding empty subunits. Bar: 1 μ m.

temperature sensation and autonomic dysfunction with preservation of tendon reflexes and large-fiber sensory modalities, prominent decrease in unmyelinated nerve fiber density (Said et al., 1983) and increase in small caliber unmyelinated fibers (Brown et al., 1976), in addition to the selective loss of small myelinated nerve fibers, were reported. We also found that the biopsies from three patients with prominent autonomic dysfunction showed significantly lower unmyelinated nerve fiber density as compared with those from 25 patients without clinical autonomic failure (Fig. 5) (Kanda et al., 1985).

(3) Amyloid neuropathy

Peripheral neuropathy is a prominent feature in primary amyloidosis (AL) and familial amyloid polyneuropathy (FAP). Pathological findings of sural nerves in these two are almost the same: relatively selective loss of unmyelinated and small myelinated nerve fibers (Dyck and Lambert, 1969; Thomas and King, 1974b; Said et al., 1984). The absence of the blood-nerve barrier in the sympathetic ganglia and dorsal root ganglia may allow access of amyloidogenic proteins into nerve parenchyma and eventually the damage to the neural structures becomes prominent, either by mechanical compression, ischemia or toxicity of amyloid protein itself. However, the mechanism of relatively severe involvement in unmyelinated nerve fibers has not been clearly elucidated.

(4) Hereditary sensory and autonomic neuropathy (HSAN)

Hereditary sensory and autonomic neuropathy, a group of inherited neuropathies with predominant involvement of sensory and autonomic neurons, includes a dominantly inherited variety (HSAN I), recessively inherited sensory neuropathy (HSAN II), familial dysautonomia (HSAN III), hereditary anhidrotic sensory neuropathy (HSAN IV), and a congenital sensory neuropathy with selective loss of small myelinated nerve fibers (HSAN V). Although loss of unmyelinated nerve fibers has been reported in all five varieties, systematic pathological studies are still scarce because of the rarity of the disorder. In HSAN I, the decrease in unmyelinated nerve fibers was reported to be almost in parallel with that of myelinated nerve fibers (Danon and Carpenter, 1985; Dyck, 1993). HSAN II showed minimum decrease of unmyelinated nerve fibers, in contrast with severe depletion of myelinated ones (Nukada et al., 1982). A devastating decrease in unmyelinated nerve fibers with relative preservation of myelinated nerve fibers was described in HSAN III (Aguayo et al., 1971) and almost complete disappearance of unmyelinated nerve fibers was reported in HSAN IV (Goebel et al., 1980; Matsuo et al., 1981). Recently, a deletion-, splice- and missense-mutation in the tyrosine kinase domain of the genomic DNA encoding TRKA were reported in three unrelated HSAN IV patients (Indo et al., 1996); this suggests that

the NGF-TRKA system has a crucial role in the development of healthy unmyelinated nerve fibers. The decrease in unmyelinated nerve fibers was less severe in HSAN V (Dyck et al., 1983).

(5) Fabry disease

The accumulation of globotriaosylceramide (CTH) in the dorsal root ganglia and autonomic ganglia is believed to cause peripheral neuropathy in this X-linked hereditary disorder. Decrease in unmyelinated nerve fibers and small myelinated nerve fibers was reported (Ohnishi and Dyck, 1974); however, some authors described an increase in small unmyelinated nerve fibers without apparent decrease in its density (Sima and Robertson, 1978; Toyooka and Said, 1997) and Fukuhara et al. (1995) did not find any abnormality in un-myelinated nerve fibers. The discrepancy of pathological pictures among patients might be related to the difference in the amount of CTH accumulated in the peripheral nervous system. A heterozygote female may develop neuropathy due to CTH accumulation, and a patient with decreased unmyelinated nerve fiber density was reported (Mutoh et al., 1988).

(6) Xeroderma pigmentosum

Group A xeroderma pigmentosum (XP), the commonest form of XP in Japan, corresponds to the most severe clinical form of XP. Most of the patients give rise to serious involvement of the central and peripheral nervous system in addition to cutaneous lesions; this combination comprising the De Sanctis Cacchione syndrome. We scrutinized the PNS pathology in two patients and found a severe depletion of unmyelinated nerve fibers in both of them (Kanda et al., 1990).

The peripheral neuropathies with substantial involvement of unmyelinated nerve fibers can be divided into three categories: (1) hereditary degenerative disorder involving dorsal root ganglia and/or sympathetic ganglia (e.g., HSAN, XP); (2) acquired disorder with prominent involvement of postganglionic sympathetic fibers (e.g., acute pandysautonomic neuropathy); and (3) a group of systemic disorders in which some "local" factors are considered to be related to the pathogenesis of neuropathy (e.g., amyloidosis: deposition of amyloid protein; Fabry disease: CTH accumulation; diabetic neuropathy: scattered ischemic lesions and metabolic derangement in Schwann cells). Distal axonopathy, the most common form of myelinated nerve fiber depletion in the distal nerve trunks such as sural nerves, is usually not conspicuous in unmyelinated nerve fibers.

Peripheral neuropathy where unmyelinated nerve fiber changes has been documented

Because even the pathological report of peripheral

neuropathy does not necessarily mention the changes in unmyelinated nerve fibers, the knowledge concerning the unmyelinated nerve pathology in individual disorders is still not enough.

(1) Alcoholic neuropathy

There is still controversy about whether the "alcoholic" neuropathy is due to a direct toxic effect of alcohol, to malnutrition, or both. The pathological picture is that of so-called "dying back neuropathy", so unmyelinated nerve fibers are usually much less affected as compared with myelinated nerve fibers. However, moderate reduction of unmyelinated nerve fibers is described in patients complaining of severe paresthesia of extremities (Takahashi, 1984). Alcoholic neuropathy is one of the major causes of sporadic acrodystrophic neuropathy. In alcoholic acrodystrophic neuropathy patients, the large myelinated fibers are primarily involved, followed by small myelinated and unmyelinated fibers (Said, 1980). Although acrodystrophy is essentially due to the severe derangement of primary sensory neurons, the depletion of unmyelinated nerve fibers in this disorder may partly be related to the autonomic nervous system involvement.

(2) Toxic neuropathy

No toxic neuropathies with selective loss of unmyelinated nerve fibers have been reported. Most of the toxic neuropathy shows the pathological picture of dying back neuropathy and unmyelinated nerve fibers are usually least affected. Unmyelinated nerve fiber changes were reported in neuropathies due to isoniazid (Ochoa, 1970), thalidomide (Schroder and Gibbels, 1977), sodium cyanate (Ohnishi et al., 1975), n-hexane (Vallat et al., 1981), ethylene oxide (Kuzuhara et al., 1983), and almitrine (Gherardi et al., 1987) intoxication.

(3) Chronic inflammatory demyelinating polyradiculoneuropathy (CIDP)

CIDP is an autoimmune disorder targeting myelin component of peripheral nerve; hence, theoretically, this disorder does not involve unmyelinated nerve fibers (Prineas and McLeod, 1976; Kanda et al., 1991a). However, Gibbels and Kentenich (1990) found primary involvement of unmyelinated nerve fibers in all 7 cases they analyzed and mentioned that the severity of their changes were in parallel with the degree of demyelination. In our experience, cases with severely depleted myelinated nerve fibers usually showed some morphological changes in unmyelinated nerve fibers (unpublished observation). The possible explanations for the involvement of unmyelinated nerve fibers in CIDP are: (1) damage by humoral factors which attack unmyelinated nerve fibers and/or their Schwann cells, and (2) mechanical compression by the proximally situated inflammatory lesions.

(4) Paraproteinemic neuropathy

Like CIDP, this is also an autoimmune disorder targeting myelin component of peripheral nerve; hence, this disorder is believed not to involve unmyelinated nerve fibers (Swash et al., 1979; Smith et al., 1983). Conversely, we found a severe reduction of unmyelinated nerve fiber density in biopsied sural nerve in a patient with Waldenström's macroglobulinemia whose IgM showed anti-myelin-associated glycoprotein (MAG) and sulfoglucuronosyl paragloboside (SGPG) activity (Kanda et al., 1998b). Vital et al. (1989) described marked damage to unmyelinated nerve fibers in 30 of 31 superficial peroneal nerve specimens. Serum in 25 of their cases showed anti-MAG activity. Mata et al. (1988) demonstrated the deposition of immuno-globulin throughout the compact myelin in this disorder and, to a lesser extent, within the Schwann cells. Surface antigens reacting with anti-MAG/SGPG IgM antibody have been described in cultured human Schwann cells (Jauberteau et al., 1992). Thus, direct immunological insult to Schwann cells sustaining unmyelinated nerve fibers may be a cause of unmyelinated nerve fiber loss in this



Fig. 6. Pure autonomic failure (61F). Cluster of collagen pockets (arrow) is occasionally observed. Bar: 1 μ m.

disorder, in addition to the mechanical effects of tumor cell infiltrates and/or inflammatory lesions scattered in the endoneurium.

Changes of unmyelinated nerve fibers in degenerative disorders of central nervous system

(1) Amyotrophic lateral sclerosis (ALS)

Although ALS has long been considered to be a disorder in which lesion is confined to the primary and secondary motor neurons, the involvement of the sensory peripheral nervous system has been proved in some articles (Dyck et al., 1975; Kawamura et al., 1981; Bradley et al., 1983). Bradley et al. (1983) described a reduced number of unmyelinated nerve fibers in the 4 postmortem specimens of ALS. They also showed a significantly greater number of denervated Remak cell processes (empty Schwann cell subunit) in both ALS biopsy and postmortem specimens compared with the controls. Ben Hamida et al. (1987) described the reduction of small unmyelinated nerve fibers using nine samples of biopsied superficial peroneal nerve. We analyzed the quantitative changes in unmyelinated nerve fibers of sural nerves in 11 autopsy cases of ALS. Ordinary ALS patients demonstrated no involvement; however, the cases with long survival due to the application of ventilatory support showed a bimodality in diameter histogram of unmyelinated nerve fibers, and a patient with involvement of systems other than motor pathways showed an abnormal value in two indices: a low percentage of subunits containing axon(s) and a high mean number of Schwann cell profiles per axon (Kanda et al., 1996).

(2) Parkinson disease

CNS degenerative disorders which cause peripheral neuropathy are numerous but do not include Parkinson disease. Autonomic deficits such as orthostatic hypotension are not uncommon clinical features in this disorder; however, many authors suggested that the autonomic disturbances in Parkinson disease may be due to a lesion at a higher level in the nervous system above the medulla or in the hypothalamus (Appenzeller and Goss, 1971). So far, our publication (Kanda et al., 1996) is the only one which has handled the pathological changes of peripheral nervous system in Parkinson disease. In this study, unmyelinated axons and their Schwann cells presented no specific qualitative changes in the majority of patients. However, a significant reduction of the mean value of unmyelinated nerve fiber density (21%) was found in Parkinsonian patients (Kanda et al., 1996).

(3) Multiple system atrophy (MSA)

In contrast to the two disorders described above, the presence of peripheral neuropathy in MSA is widely

accepted. The majority of previous articles have been concerned with changes in myelinated nerve fibers, and histological examinations using biopsied materials have shown a preferential reduction in large myelinated nerve fibers (Galassi et al., 1982; Rossi et al., 1986). Few articles have described the changes of unmyelinated nerve fibers in MSA. Galassi et al. (1982) stated that there was no abnormality compared with the values published for controls. In our study using 4 autopsy cases, the mean value of unmyelinated nerve fiber density was significantly decreased (23%), and this decrease almost paralleled that of myelinated nerve fiber density. Bimodal patterns of the diameter histogram of unmyelinated axons were observed in two older (63F and 73F) patients (Kanda et al., 1996).

(4) Pure autonomic failure (PAF)

The exact site of autonomic nervous system involvement in this rare disorder is still in dispute. Some postmortem reports from PAF patients maintain that degeneration primarily affects the preganglionic sympathetic nervous system (Johnson et al., 1966). However, physiological studies suggest predominant postganglionic sympathetic involvement, and several pathological reports have demonstrated more significant involvement in sympathetic ganglia than in intermedio-lateral columns (van Ingelghem et al., 1994). Differing from acute pandysautonomia, PAF does not involve primary sensory neurons clinically or electrophysiologically. Therefore, the precise evaluation of the pathological picture in the sural nerve of PAF patients may clear up the unsolved problems, including: (1) the exact percentage of primary afferents and sympathetic postganglionic fibers in human sural nerve, and (2) the topographic distribution of these two components. We examined biopsied sural nerve specimens obtained from seven Japanese PAF patients (Kanda et al., 1998a). The mean unmyelinated nerve fiber density in these patients was 40% less than in age-matched controls. Increased numbers of clusters of collagen pockets not containing unmyelinated axons were the most prominent findings in PAF (Fig. 6). This appears to reflect recent dropout of a group of sympathetic efferents and suggests grouping of unmyelinated fibers by modality at the level of the sural nerve trunk.

(5) Hereditary spinocerebellar degeneration (SCD)

Classification of inherited SCD is now based on their genetic features, which supplement, clarify, and sometimes replace the previous clinical and pathological descriptions in this group of disorders. Peripheral nerve pathology in each genetically-confirmed group is still obscure except in two SCDs in which peripheral neuropathy is included as one of the main clinical features: Friedreich ataxia (Said et al., 1986) and Machado-Joseph disease (Coutinho et al., 1986; Kanda et al., 1989). Said et al. (1986) analyzed 8 biopsied sural

nerves in Friedreich's ataxia and described normal unmyelinated nerve fibers in all of them, even in cases with prominent disappearance of large myelinated nerve fibers. This pathological picture corresponds to that of "dying-back neuropathy". In non-Friedreich hereditary SCD, McLeod (McLeod and Evans, 1981) scrutinized 4 biopsies and stated that the density and fiber diameter distribution were similar to those in control nerves.

Conclusion

In usual cases of peripheral neuropathy, pathological alteration of nerve fibers becomes prominent in the following order: first, large myelinated nerve fibers; second, small myelinated nerve fibers; and finally, unmyelinated nerve fibers. Hence, severe involvement of unmyelinated nerve fibers with relative sparing of large myelinated ones means substantial diagnostic value. Although electron microscopic investigation is necessary due to their tiny size, and that a morphometric analysis is required to evaluate the quantitative change of unmyelinated fibers (densities) because of uneven distribution of unmyelinated nerve fibers in the endoneurium, the morphological evaluation of unmyelinated nerve fibers is not such a difficult job. For the complete analysis of sural nerves obtained at biopsy and even at autopsy, qualitative and quantitative estimation of unmyelinated nerve fibers should be performed in all specimens. The author hopes this review is helpful for the readers.

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