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Subperineurial Space of the Sural Nerve in Various Peripheral Nerve Diseases

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Summary. The size of the subperineurial space of the sural nerve has been evaluated quantitatively in 69 cases of various peripheral nerve diseases and in controls. A significant increase was found in beriberi neuropathy (6 cases) and idiopathic polyradiculoneuropathy (9 cases) as compared with the control (8 cases). On electron microscopy a few macrophages, fibroblast processes, collagen fibrils with a diameter of 50 nm, microfibrils with a diameter of 8 nm, and amorphous material were observed in both the enlarged subperineurial space and the endoneurial intercellular space. They were less frequently observed in controls. No significant correlation was found between the size of subperineurial space and the density of myelinated fibers.

Key words: Subperineurial space – Peripheral neuropathy – Sural nerve

Although enlargement of the subperineurial space of peripheral nerve has been noted in acute and chronic idiopathic polyneuritis and chronic relapsing polyneuritis (Harris and Newcomb, 1929; Austin, 1958; Matthews et al., 1970; Prineas, 1972; Prineas and McLeod, 1976), no systematic quantitative study on the size of subperineurial space in various peripheral nerve diseases has been reported. We quantified the size of subperineurial space in 69 sural nerves to establish in which diseases the subperineurial space is increased. In addition, the relationship between the size of subperineurial space and the density of myelinated fibers was analyzed.

Materials and Methods

The sural nerve was obtained at biopsy in 6 cases of beriberi neuropathy, 8 cases of idiopathic polyradiculoneuropathy, 5 cases of diabetic neuropathy, 10 cases of toxic neuropathy (5 cases of clioquinol neuropathy, one case of arsenical neuropathy, 2 cases of n-hexane neuropathy, one case of organophosphate insecticide (Dipterex) neuropathy, and one case of thiamphenicol neuropathy), 12 cases of heredodegenerative diseases (4 cases of spinocerebellar degeneration, 2 cases of familial spastic spinal paralysis, 5 cases of Charcot-Marie-Tooth disease, and one case of hereditary sensory neuropathy), 7 other miscellaneous neuropathies (one case of each of alcoholic neuropathy, neuropathy with malabsorption syndrome, neuropathy with polyarteritis nodosa, tabes dorsalis with diabetes mellitus, von Recklinghausen's disease, familial amyloid neuropathy, and neuropathy with herpes zoster), and obtained at autopsy in each of 13 cases of malignancy (3 cases with neuropathy), and of 8 cases of sudden death without peripheral nerve involvement as the controls.

The sural nerves were fixed with 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 2 h. The tissue was washed in the same buffer, postfixed in 1% osmium tetroxide in the same buffer for 2 h, dehydrated, and embedded in epoxy resin.

Photographic enlargements (× 130) of transverse epoxy sections stained with toluidine blue were used for the determination of the size of the subperineurial space. The size of subperineurial space was expressed as the mean ratio (%) of the space devoid of nerve fibers and Schwann cells just beneath the perineurium, to the endoneurial area surrounded by the innermost layer of the perineurium in each of the three to seven fascicles of the sural nerve in each case (Figs. 1 and 2). It was concluded that the size of subperineurial space was not significantly altered with the time elapsed between fixation and death in preliminary studies of biopsy and autopsy materials. Photographic enlargements (× 1000) of transverse epoxy sections were used for the determination of the density (numbers per square millimeter of fascicular area) of the myelinated fibers of the sural nerve in each case. The mean size of subperineurial space and mean density of the myelinated fibers were obtained in each of the groups of beriberi neuropathy, idiopathic polyradiculoneuropathy, diabetic neuropathy, toxic neuropathy, heredodegenerative diseases, other miscellaneous neuropathies, malignancy and control. In addition, the mean size of subperineurial space was also calculated in three groups, the group with a myelinated fiber density of more than 8000/mm^2, with a myelinated fiber density between 8000/mm^2 and 5000/mm^2, and with a myelinated fiber density of less than 5000/mm^2.

Thin sections were cut on a microtome, stained with uranyl acetate and lead citrate and examined with an electron microscope,

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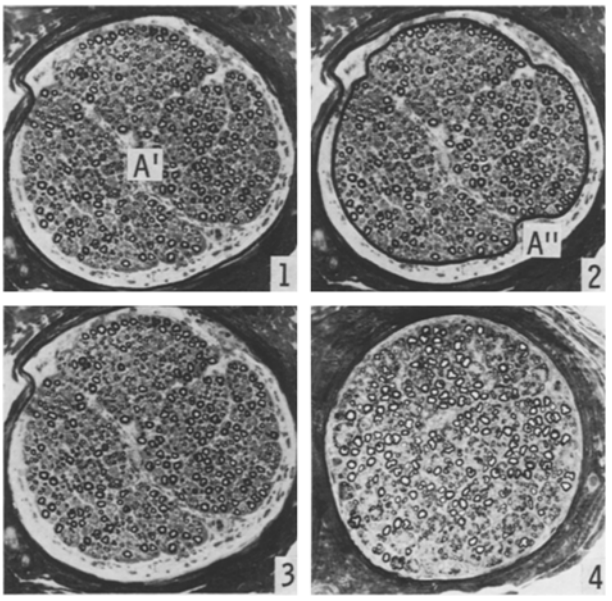

Figs. 1 and 2. The size of subperineurial space (SPS) was defined as

$$SPS = \frac{\text{subperineurial space (A'')}}{\text{endoneurial space (A')}} \times 100$$

Fig. 3. Enlarged SPS in a case of beriberi neuropathy (× 98)

Fig. 4. The normal range size of SPS in a control case. Epon embedded transverse sections stained by toluidine blue (× 98)

paying a special attention to the contents of the enlarged subperineurial space and endoneurial intercellular space. In three cases of beriberi neuropathy and seven cases of idiopathic polyradiculoneuropathy with an enlarged subperineurial space, paraffin embedded sections were stained with hematoxylin and eosin, Alcian blue, and the periodic-acid Schiff method.

Results

The mean size of subperineurial space and mean myelinated fiber density of each of 8 groups are summarized in Table 1. The mean size of subperineurial space was significantly larger in beriberi neuropathy ($P<0.001$) and in idiopathic polyradiculoneuropathy ($P<0.05$) as compared with the controls (Figs. 3 and 4). It was not significantly different between each of the groups of diabetic neuropathy, toxic neuropathy, heredodegenerative disease, other miscellaneous neuropathies and malignancy, and the control. In beriberi neuropathy it was significantly larger than in any other groups. The size of the subperineurial space and myelinated fiber density in each of cases of beriberi neuropathy and idiopathic polyradiculoneuropathy are shown in Tables 2 and 3.

On light microscopy of Epon embedded sections, a few cellular components and amorphous material lightly stained with toluidine blue were identified (Fig. 3). Periodic acid-Schiff staining for the material in

Table 1. Size of subperineurial space (SPS) and myelinated fiber density (FD) in peripheral nerve diseases

	Size of SPS[a]	FD[b]	Number of cases
Control	7.13 ± 0.58	8,659 ± 726	8
Beriberi neuropathy	18.12 ± 2.23	6,804 + 841	6
Idiopathic polyradiculoneuropathy	9.79 ± 0.85	10,317 ± 1,616	8
Diabetic neuropathy	6.32 ± 0.97	5,758 ± 1,150	5
Toxic neuropathy	7.88 ± 0.68	8,693 ± 603	10
Heredodegenerative disease	7.29 ± 0.63	6,172 ± 1,499	12
Other neuropathies	9.00 ± 1.14	4,894 ± 929	7
Malignancy	6.70 ± 0.75	7,981 ± 1,213	13

[a] expressed by %, Mean ± SE
[b] Number/mm^2 of transverse fascicular area

Table 2. Size of subperineurial space (SPS) and myelinated fiber density (FD) in beriberi neuropathy

Case	Size of SPS	FD	Duration of neurological symptoms at biopsy	Pedal edema at biopsy	Cardiomegaly in the clinical course
1. 17Y, male	27.5	8109	2 weeks	+	+
2. 18Y, male	18.5	7423	3 weeks	+	unknown
3. 18Y, male	15.0	3813	1 months	+	+
4. 17Y, male	15.6	5023	2 months	−	+
5. 19Y, male	11.8	9446	2 months	−	+
6. 15Y, male	20.3	7011	2.5 months	−	unknown

Table 3. Size of subperineurial space (SPS) and myelinated fiber density (FD) in polyradiculoneuropathy

	Size of SPS		Duration of neurological symptoms at biopsy
1. 19Y, male	9.1	9060	1 M
2. 24Y, male	9.5	14,102	1.5 M
3. 13Y, male	8.2	12,222	1.5 M
4. 54Y, male	14.0	12,479	3.5 M
5. 27Y, male	7.0	11,629	6 M
6. 45Y, female	12.9	129	10 M
7. 3Y, female	8.8	14,087	2 Y
8. 4Y, male	8.8	8828	3 Y 4 M

Table 4. Myelinated fiber density (FD) and size of subperineurial space (SPS)

Myelinated fiber density (Number/mm^2)		Size of SPS (%, Mean ± SE)
FD ≧ 8000	(33 cases)	9.08 ± 0.68
5000 ≦ FD < 8000	(21 cases)	8.55 ± 1.02
FD < 5000	(15 cases)	7.51 ± 0.96

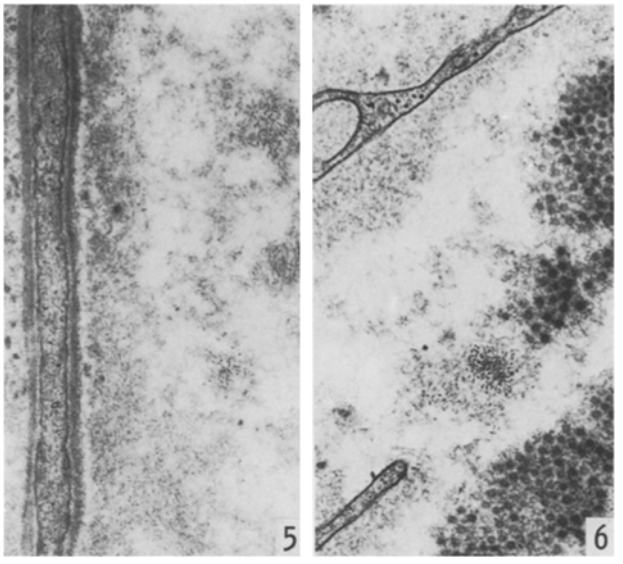

Fig. 5. Microfibrils with a diameter of 8 nm and amorphous material beneath the perineurium in a case of beriberi neuropathy (× 20,000)

Fig. 6. Microfibrils with a diameter of 8 nm, collagen fibrils with a diameter of 50 nm, amorphous material and fibroblast processes in the subperineurial space from a case of beriberi neuropathy (× 20,000)

subperineurial space and in the endoneurial intercellular space was negative in all cases examined of beriberi neuropathy and idiopathic polyradiculoneuropathy. Alcian blue staining for the material in subperineurial space and in the endoneurial intercellular space was slightly positive in two cases (cases 7 and 8 in Table 3) out of seven cases of idiopathic polyradiculoneuropathy.

On electron microscopy in three cases of beriberi neuropathy and two cases of idiopathic polyradiculoneuropathy with a definitely enlarged subperineurial space, a few macrophages, fibroblast processes, collagen fibrils with a diameter of 50 nm and microfibrils with a diameter of 8 nm were observed in both the enlarged subperineurial space and the endoneurial intercellular space (Figs. 5 and 6). These structures were also observed in controls in both the subperineurial space and the endoneurial intercellular space, although they were much less evident.

The mean size of the subperineurial space in three groups with different myelinated fiber density is shown in Table 4. No significant difference in the size of the subperineurial space was found between three groups.

Discussion

The quantitative study of the size of the subperineurial space in this report has confirmed the enlargement of subperineurial space in idiopathic polyradiculoneuropathy reported in the literature (Harris and Newcomb, 1929; Austin, 1958; Matthews et al., 1970; Prineas, 1972; Prineas and McLeod, 1976). Among eight cases of idiopathic polyradiculoneuropathy, no correlation was found between the size of the subperineurial space and the duration of neurological symptoms at biopsy or the myelinated fiber density (Table 3). In beriberi neuropathy the prominent increase of the subperineurial space has rarely been mentioned in the literature. Our data indicate that subperineurial edema exists in the sural nerve irrespective of the presence or absence of edema at biopsy in beriberi neuropathy. The degree of enlargement of the subperineurial space was maximal in beriberi neuropathy amongst the eight groups studied. No definite correlation was found between the size of the subperineurial space and the duration of neurological symptoms at biopsy among the six cases of beriberi neuropathy.

On electron microscopy, a few macrophages, fibroblasts, collagen fibrils, 8 nm microfibrils and amorpous material, which were also found in controls, were found in both idiopathic polyradiculoneuropathy and beriberi neuropathy. No aggregations of acid mucopolysaccharide-like material, which were reported by Prineas (1973) in acute polyradiculoneuropathy, were observed in either idiopathic polyradiculoneuropathy or beriberi neuropathy.

In studies on experimental neuropathy, enlargement of subperineurial space has commonly been seen in experimental Wallerian degeneration (Nichols et al., 1968), lead neuropathy (Ohnishi et al., 1977), hexacarbon neuropathy (Spencer and Schaumburg, 1977), and neuropathy due to p-bromophenylacetylurea (Ohnishi, unpublished data) and methylmercury intoxication (Ohnishi, unpublished data).

We assume that the enlargement of the subperineurial space can be best explained as a secondary result of nerve degeneration related to various different etiologies. It may be related with acuteness of the fiber degeneration and the nature or the pathogenic mechanism of the fiber degeneration. In idiopathic polyradiculoneuropathy, alterations in vascular permeability due to allergic or inflammatory reactions may be partly responsible for the enlargement of subperineurial space. In beriberi neuropathy, on the other hand, the subcutaneous edema in lower limbs and the enlargement of the subperineurial space may have occurred as a consequence of metabolic derangements in capillaries. Increased extracellular fluid in the endoneurium may have adversive effects on nerve fibers.

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References

Austin, J. H.: Recurrent polyneuropathies and their corticosteroid treatment. With five-year observations of a placebo-controlled case treated with corticotrophin, cortisone, and prednisone. Brain **81**, 157–192 (1958)

Harris, W., Newcomb, W. D.: A case of relapsing interstitial hypertrophic polyneuritis. Brain **52**, 108–116 (1929)

Matthews, W. B., Howell, D. A., Hughes, R. C.: Relapsing corticosteroid-dependent polyneuritis. J. Neurol. Neurosurg. Psychiatry **33**, 330–337 (1970)

Nichols, P. C., Dyck, P. J., Miller, D. R.: Experimental hypertrophic neuropathy: Changes in fascicular area and fiber spectrum after acute crush injury. Mayo Clin. Proc. **43**, 297–305 (1968)

Ohnishi, A., Schilling, K., Brimijoin, W. S., Lambert, E. H., Fairbanks, V. F., Dyck, P. J.: Lead neuropathy. 1. Morphometry, nerve conduction, and choline acetyltransferase transport: new finding of endoneurial edema associated with segmental demyelination. J. Neuropathol. Exp. Neurol. **36**, 499–518 (1977)

Prineas, J. W.: Acute idiopathic polyneuritis – An electron microscope study. Lab. Invest. **26**, 133–147 (1972)

Prineas, J. W., McLeod, J. G.: Chronic relapsing polyneuritis. J. Neurol. Sci. **27**, 427–458 (1976)

Spencer, P. S., Schaumburg, H. H.: Ultrastructural studies of the dying-back process. IV. Differential vulnerability of PNS and CNS fibers in experimental central-peripheral distal axonopathies. J. Neuropathol. Exp. Neurol. **36**, 300–320 (1977)

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