Peripheral neuropathy in the course of progressive systemic sclerosis: light and ultrastructural study.

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We present the case of a woman with progressive systemic sclerosis (PSS) in whom the usual symptoms were preceded by a rapidly progressive peripheral neuropathy. Few cases of peripheral nerve involvement have been described. For the first time we report an ultrastructural study of an affected peripheral nerve and muscle.

In the sural nerve we found an almost complete loss of myelinated fibers. Schwann cells showed an abnormal hyperplasia of their basal membranes and structural signs of denervation. Spindle-shaped banded structures were seen in the cytoplasm of Schwann cells and in the endoneurium. On the basis of these ultrastructural data some hypotheses on the pathogenetic mechanism of this neuropathy are discussed.

Key-Words: progressive systemic sclerosis — peripheral nervous system — electron microscopy.

Introduction

Progressive Systemic Sclerosis (PSS) is a chronic inflammatory disease of the connective tissue, which affects the skin and other organs and is characterized by an abnormal deposition of collagen and by vascular lesions of the capillaries and the small vessels. Clinically, the organs most commonly affected are skin, lungs, heart, kidneys, the gastrointestinal tract and the osteoarticular system [17].

Involvement of the peripheral nervous system (PNS) in the course of PSS is not a common feature and very few cases of peripheral neuropathies have been reported so far [2, 6, 8, 9, 13, 18]. Only in two cases [13, 18] has a pathological study of this neuropathy been performed with light microscopy techniques and no electron microscopy studies have reported. The present work describes a light and ultrastructural study of the tibialis anterior muscle and sural nerve of a patient with PSS and clinical PNS involvement.

Case Report

A 63-year-old white woman suffering from left leg myalgias came to our attention in April 1978. Her family and past medical histories were unremarkable. She had started having attacks of pain in her left leg and foot in July 1977. In fall 1977 she had been admitted to a general hospital, where a mild hypochromic anemia, a slightly reduced α_1 and α_2 globulin value and a threefold increase of the erythrocyte sedimentation rate were detected.

In April 1978, when she was admitted to the neurological clinic of our university, she was found to have hypotrophy of the left leg muscles, with elementary muscular strength rated from 2 to 3 in different muscles (on an 0 to 5 scale) on the affected side and normal strength (rated 5) on the right. There was also a bilateral hypoesthesia for light touch, pain and vibration localized distally on both legs, with no specific topographical arrangement. Muscle-stretch reflexes were very weak in the lower limbs, while the left

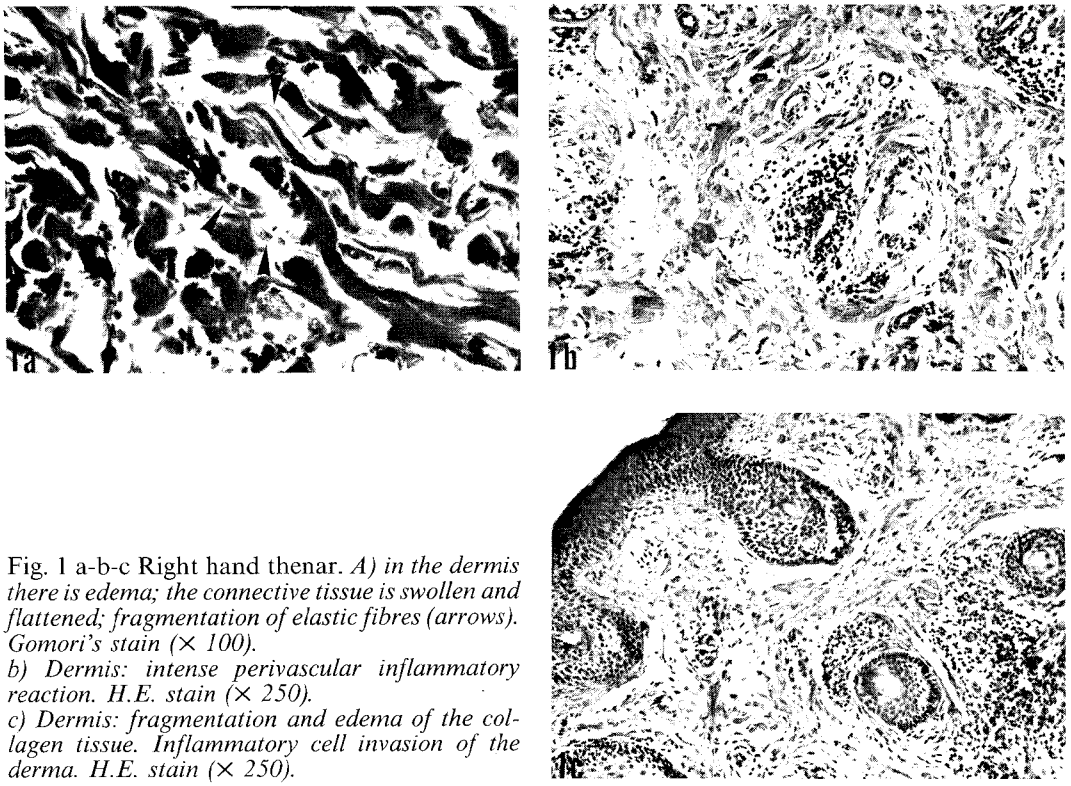
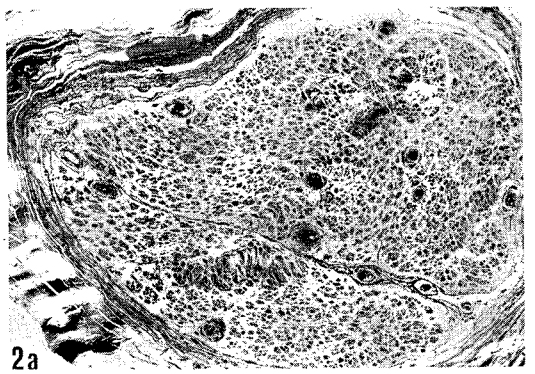
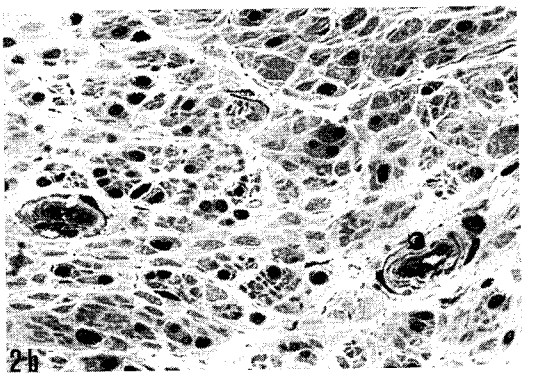


Fig. 1 a-b-c Right hand thenar. *A) in the dermis there is edema; the connective tissue is swollen and flattened; fragmentation of elastic fibres (arrows). Gomori's stain (× 100).*
b) Dermis: intense perivascular inflammatory reaction. H.E. stain (× 250).
c) Dermis: fragmentation and edema of the collagen tissue. Inflammatory cell invasion of the derma. H.E. stain (× 250).

outer hamstring reflex was absent. The clinical examination was otherwise normal. Electromyography showed a pattern of total denervation of the first dorsal interosseous muscle of both feet; the voluntary activation pattern was reduced both in the left tibialis anterior and in the right peroneus tertius. The motor conduction velocity was normal. Corticosteroid treatment (prednisolone acetate) apparently resulted in an improvement of muscular strength. During the following years the patient's symptoms progressively worsened and she was admitted several times to our clinic. The major features of this course were:
June 1978: Intense arthro-myalgias of all four limbs; the neuropathy became bilateral. X-ray of chest and digestive system showed initial sclerosis of the left lung and reduced motility of the esophagus.
September 1978: The muscular atrophy became massive, elementary voluntary strength averaged 2 bilaterally in the lower and 3 in the upper limbs. The motor conduction velocity of the ulnar nerve was extremely reduced (19 m/sec.). During her stay in the clinic the patient suffered from hypertensive crises, with blood pressure values up to 240/150 mm Hg. A positive Waaler-Rose reaction and a high serum value of the rheumatoid factor were found. A muscle and nerve biopsy were carried out (see below). Indirect immunofluorescence tests were performed on serum samples tested against brain, striated muscle, liver, kidney and aorta slices in order to detect the presence of specific serum antibodies; only an IgG specific anti-elastic fibre reactivity was present.

May 1979: After treatment with corticosteroids, vitamins and a high-calorie diet the muscular strength seemed to improve. But there was no improvement in the sensory disturbances: the patient reported difficulty in swallowing. The skin appeared to be waxy, hard and erythematous, especially on the distal portions of the four limbs and on the face. Teleangiectasias were diffusely present on the neck and chest. Raynaud's phenomenon was not seen. A gastroscopic examination showed marked reduction of peristalsis in the lower third of the esophagus. A skin biopsy was performed. Blood tests showed only a reduction of the C_3 complement fraction and clearly positive Waaler-Rose reaction and rheumatoid factor.
During the course of the disease the following





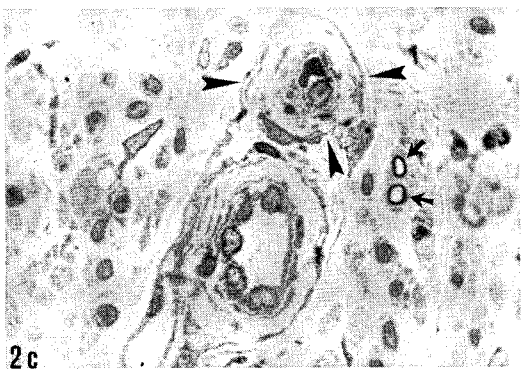


Fig. 2 a-b-c Semithin sections. a) Sural nerve: *total loss of myelinated fibres. Toluidine blue stain. (× 120).*
b) Sural nerve: detail of figure 2A. (× 400).
c) Sural nerve: severe vascular alterations, endothelial edema and initial stenosis. (head arrows). Isolated myelinated fibres (arrows). Toluidine blue stain. (× 1000).

laboratory tests remained normal: serum tests for syphilis, L.E. cell test, C.E.A. test, anti-DNA factors, E rosette test, PHA blast formation test, serum and urine anti-α-ϒ-k- -μ chains immunoelectrophoresis.
The patient's clinical condition remained stable until early February 1980 when acute renal failure, atrial fibrillation and high fever set in and she died at home within a few days.

Materials and methods

Skin, nerve and muscle biopsy specimens were embedded in paraffin for microscopic examination. Several stains were used to evaluate connective tissue and cellular alterations: hematoxylin eosin, periodic acid Schiff, Gomori and Masson trichrome and thioflavine-T stain. Multiple specimens for electron microscopy were taken from the sural nerve and the tibialis anterior muscle of the left side. Specimens were rapidly fixed in 2,5-glutaraldehyde 0,1 M phosphate buffered at pH 7.4 and postfixed in Dalton chrome-osmium, they were dehydrated in alcohol series and cleared in propylene oxide. Semithin sections were stained with toluidine-blue at pH 8.8 and ultrathin sections were stained with uranyl acetate and lead citrate. The ultrastructural examination was carried out by means of a Philips 300 electron microscope.

Results

Light microscopy

Skin biopsy: A specimen of the right hand thenar showed thin epithelium with reduction of the papillae. In the dermis, mainly at the level of the papillae, there was edema and abundance of vessels and collagen tissue. Sections stained for the connective tissue showed swollen and flattened fibres and edema. Moreover, the vessels located in the more superficial layers of the dermis showed inflammatory cell invasion and thickening of the wall. (Fig. 1).

Muscle biopsy: In the tibialis anterior muscle most fibres were very thin, with centrally located, packed nuclei, and showed an abnormal striation and features of mucoid degeneration. Blood vessels showed thickening of the intima and edema of the adventitia.

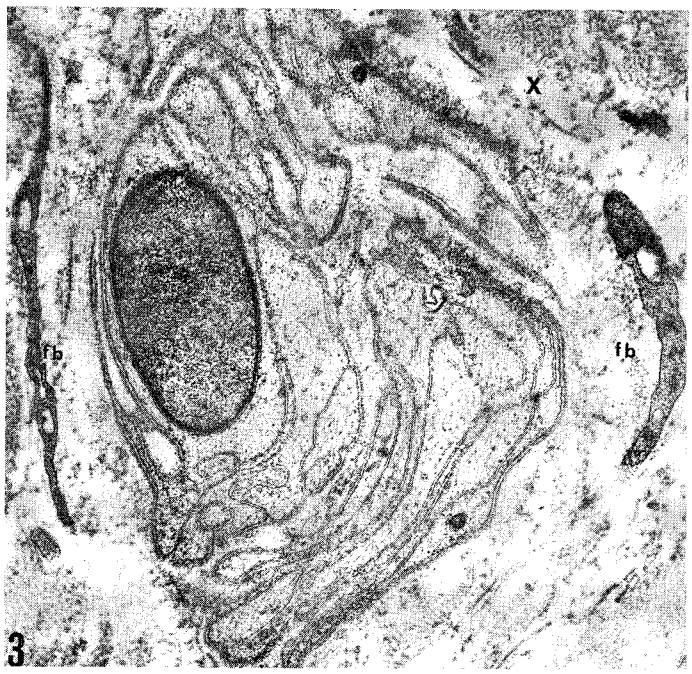


Fig. 2. Sural nerve: *hyperplasia of the Schwann cell basal membrane and presence of amorphous endoneural material (X); fb: fibroblast.*

Nerve biopsy: The sural nerve showed a reduction of myelinated fibres and increase of connective tissue in the endoneurium and perineurium. In the vasa nervorum there was thickening of the intima and edema in the adventitia. Semithin sections of the same nerve confirmed these features; there was no evidence of onion bulbs, clusters or features of degeneration of the nerve fibres (Fig. 2).

Electron microscopy

In the muscle, due to discontinuity of the myofibrillae, the normal aspect was altered in all sections. This fragmentation was often associated with storage of glycogen particles and presence of altered mitochondria and vacuoles and centrally located nuclei. In the sarcolemma no alterations were seen.
The ultrastructural study of peripheral nerve confirmed the light microscopy findings, i.e. an almost complete loss of myelinated fibres. Furthermore, the Schwann cell basal membranes were hyperplastic (Fig. 3) and the cells themselves displayed an increased number of filaments, and active endoplasmic reticulum (Fig. 4), and an increased number of Reich granules and structural signs of denervation.
Sometimes we observed in the cytoplasm of Schwann cells images of myelin structures, likely to be related to a phagic activity of these cells. The number of fibroblasts was increased; in addition in all sections we found the presence of amorphous endoneural material adjacent to the basal membrane of the Schwann cells. In the endoneural spaces there were spindle-shaped banded structures (Luse bodies, 10), either isolated or adjacent to the basal membranes.
These structures were sometimes found within the cytoplasm of Schwann cells (Fig. 5). The perineural vessels showed only some adventitial edema and were often surrounded by a fibrillary substance (Fig. 6).

Discussion

Involvement of the peripheral nervous system in the course of PSS is not a common feature and very few cases have been reported so far [8, 9, 13, 18]. Beighton et al. [2] described a case of PSS associated with a neuropathy of the trigeminal nerve, while Dimitriu et al. [6] reported a case of PSS with involvement of both the central and the peripheral nervous system.
The first interesting analogy between our case and other reported cases is the early occurrence of the PNS involvement in relation to other clinical signs of the disease. In fact, in our case, as in the ones described by Richter [13], Zülch

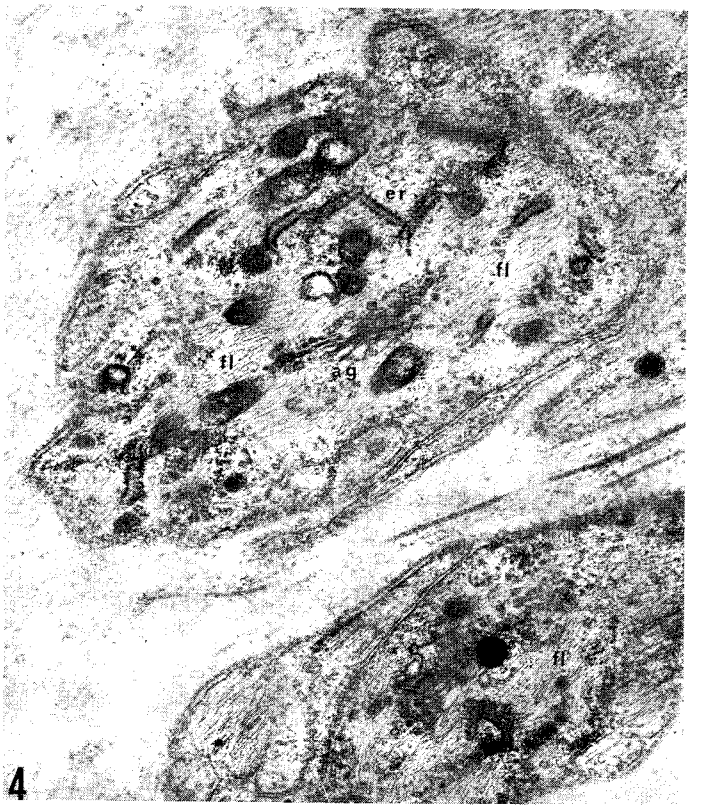


Fig. 4. Sural nerve: *increased number of filaments (fl) in the Schwann cell cytoplasm. Activation of endoplasmic reticulum (er); ag: Golgi apparatus.*

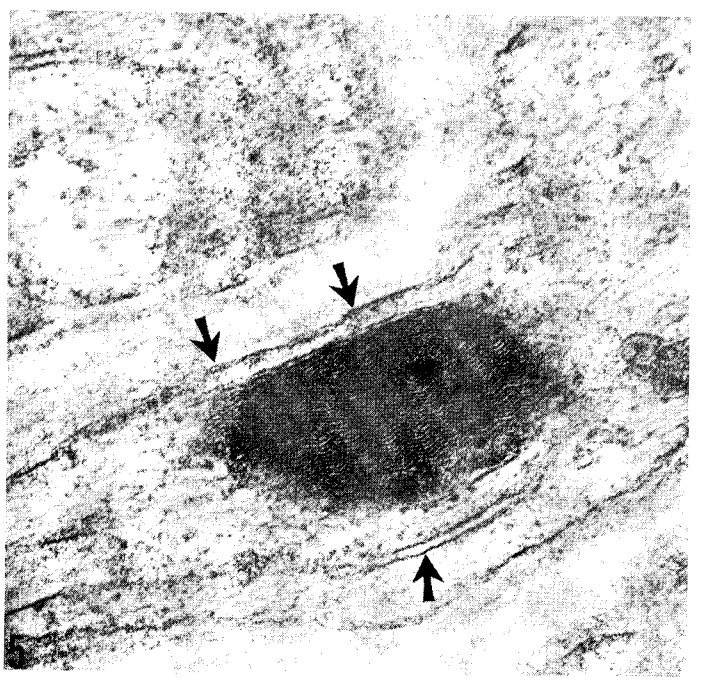


Fig. 5. Sural nerve: *Luse body located within the Schwann cell cytoplasm. The basal membrane is also displayed (arrows).*

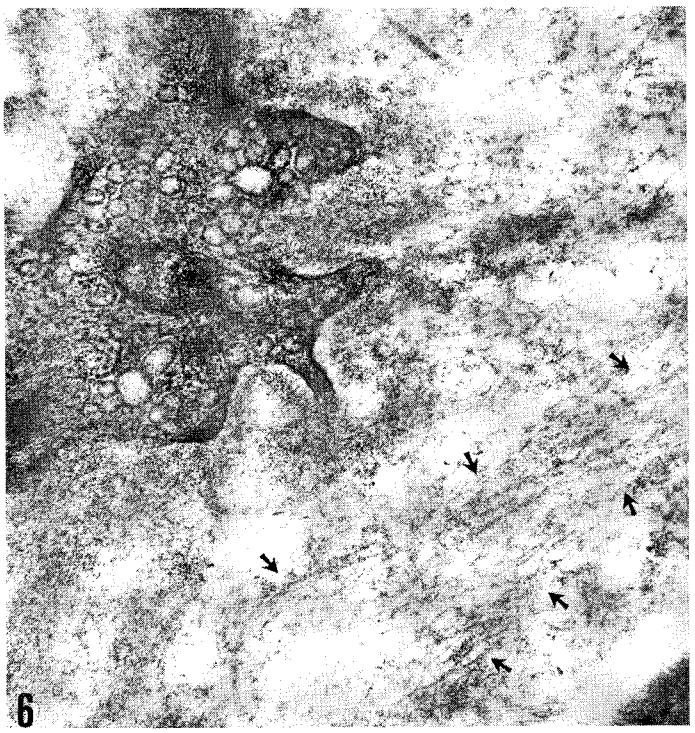


Fig. 6. Endoneural vessel: *displaying the presence of periadventitial fibrillae (arrows).*

[18] and Kibler and Rose [9], the early neurological signs were a sensori-motor mononeuropathy that later extended to the rest of the body with typical clinical and electromyographic features. The onset of the peripheral neuropathy preceded the appearance of the other clinical signs by approximately 10 months. A definite diagnosis was not reached until some months after the onset of the neuropathy, when the biopsy demonstrated clear involvement of the skin and X-ray examination substantiated the involvement of the lower third of the esophagus.

In the case of a sensori-motor neuropathy that progressively involves all four limbs and has no clear etiology, one has to look not only for a neoplasia but also for a collagen disease.

Richter [13] and Zülch [18] already described the histopathology of the peripheral neuropathy associated with PSS. In agreement with these authors, our light microscopy results can be summarized as follows:

a) increase of collagen fibre production both in the endoneurium and in the perineurium;
b) almost complete disappearance of myelinated fibres;
c) presence of an amorphous substance in the endoneurium;
d) alterations of the vasa nervorum similar to the ones found in the dermis and in the muscle; i.e. diffuse hyalinosis, proliferation of the intima and adventitial edema;
e) in the muscle a pattern of neurogenic atrophy and signs of mucoid degeneration.

For the first time we report here the ultrastructural morphology of this neuropathy. The major feature was an abnormally large amount of collagen fibres, which alter the normal fascicular structure. There were also typical aspects of denervation of the Schwann cells, characterized by an increase of the endoplasmic reticulum and filaments, associated with hyperplasia of the basal membrane. It seems that the presence of myelin fragments in the cytoplasm of the Schwann cells can be referred to a phagic activity.

Interestingly, we saw fibrillary structures either isolated or adjacent to the Schwann cell basal membranes that resemble the so-called Luse bodies [10] which have been found both in normal subjects [16] and in people with PNS neoplasms [5, 7, 10, 14]. Moreover, Pillai [12] reported that Luse bodies occurred eight months after compression of the tibialis anterior nerve of the rat only in the areas which had been compressed. Since Pillai [12] was unable to find such bodies in normal control animals, he con-

cluded that Luse bodies represented a tissue reaction to experimental compression. This hypothesis is consistent with the pathogenetic interpretation suggested by Richter [13] and Zülch [18] that the peripheral neuropathy associated with PSS is due to compression of the myelinated fibres caused by an abnormally abundant production of collagen tissue. Our findings of a large amount of collagen together with the presence of Luse bodies are consistent with this view. It must be stated, however, that the hypothesis of a mechanical compression, although suggestive, needs further support. In fact, it has still to be demonstrated that collagen production is a primary process, not dependent upon the axonal neuropathy that we have described here. Furthermore, Luse bodies have been found in several pathological conditions (as reported above) and therefore they appear not to be specific for mechanical nerve lesions. The role of vascular lesions in the pathogenesis of the neuropathy has been so far regarded as controversial because, while it can be assumed that there is a close relationship between the vascular lesions and the connective tissue pathology, the mechanism underlying it is not yet clear [13, 18]. The role of the Schwann cells in the pathogenesis of the neuropathy is also unclear. It is possible that they are responsible for the production of collagen.

It has been proposed [1, 3, 11, 15], in fact, that the Schwann cells are responsible for the abnormal production of collagen that is found during degeneration and regeneration of peripheral nerves. This has also been confirmed by *in vitro* studies which showed that cultured Schwann cells are able to produce protocollagen [4]. In conclusion, there are many open questions and it is our opinion that only comparison with further data will explain the detailed pathogenetic process of this of several other peripheral neuropathies.

Sommario

Viene presentato un caso di una paziente affetta da Sclerosi Sistemica Progressiva, caratterizzata dalla presenza di una polineuropatia ad esordio precoce accanto ai sintomi tipici della malattia. In letteratura tale associazione è stata descritta raramente. In questo lavoro viene descritto per la prima volta lo studio ultrastrutturale del nervo periferico affetto. Lo studio neuropatologico del nervo surale mostra una quasi completa perdita di fibre mieliniche. Le cellule di Schwann mostrano una anormale iperplasia della membrana basale con segni morfologici di denervazione. Di particolare interesse è la presenza di corpi di Luse (spindle-shaped banded structures) sia all'interno del citoplasma schwannico sia nell'endonevrio. Sulla base dei dati ultrastrutturali vengono discusse alcune ipotesi sul meccanismo patogenetico di questa neuropatia.

Acknowledgements. The authors are extremely grateful to Mr. A. Sbriccoli for his skilful technical assistance, to Mrs. M. Bonfante for editing and to Mr. B. Ghirotti, Mr. R. Mallucci and Mr. G.C. Rosi for the photographic documentation.

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