

## Morphological abnormalities in the sural nerve from patients with peripheral vascular disease

C. Rodríguez-Sánchez<sup>1</sup>, M. Medina Sánchez<sup>1</sup>, Rayaz A. Malik<sup>2</sup>, A.K. Ah-See<sup>2</sup> and A.K. Sharma<sup>2</sup>

<sup>1</sup>Department of Rehabilitaci3n, Hospital San Agust3n, Avil3s, Asturias and <sup>2</sup>Department of Anatomy, Marischal College, Aberdeen, UK

**Summary.** The present paper has been written in order to determine the morphological alterations in the sural nerve from patients with chronic arteriosclerotic occlusive disease. Eight patients with Peripheral Vascular Disease (PVD) and six age-matched control subjects were studied.

Morphometric data revealed two groups of patients, one of them with mild disease (n=5), and the other one with severe damage (n=3,) consisting in loss of myelinated fibres and increase in the number of small fibres ( $p < 0.005$ ).

Teased nerve fibres and electron microscopic studies also showed two types of patients, with respect to the myelin or the axonal alterations.

The unmyelinated fibre population was affected equally in both groups.

In conclusion, this study supports the idea that ischemia is able to cause structural alterations in the peripheral nerve, and that it can play a role in the development of neuropathy.

**Key words:** Ischemia, Peripheral nerve, Morphometry, Electron-microscopy

### Introduction

The term «ischemic neuritis», as used in textbooks, refers to the syndrome of pain in the lower limbs associated with peripheral vascular disease. The pain may be diffuse, being described as burning, as painful tingling, or as undue sensitivity, and may be especially bothersome at night. The pain may also be sharp and jabbing. However, these symptoms may be seen in several neuropathies and are not distinctive for the neuropathy of peripheral vascular disease (in fact, in patients with arteriosclerotic occlusive disease, there is

no direct evidence relating these symptoms to nerve fibre damage).

The frequency of occurrence of neurological deficiencies in patients with peripheral vascular disease is difficult to establish because of incomplete data in reports on this subject.

The former association was made in case reports by Oppenheim in 1893. Hutchinson and Liversedge (1956), studied 53 patients with PVD and reported several clinical neurological deficiencies. The highest rate of incidence of neurological deficiencies was reported by Eames and Lange (1967), who found such abnormalities in 88% of patients with severe vascular occlusive disease. The extent of deficiency was proportional to the ischemia. In these patients, the neurophysiological abnormalities included slowing of conduction velocity of motor fibres and increase in distal latency (Miglietta and Lowenthal, 1962; Kumlin et al., 1974). These changes were found in peroneal nerve in patients with severe peripheral vascular disease. Chopra and Hurwitz (1968, 1969a,b) also examined nerve conduction of motor fibres in media, ulnar, peroneal and femoral nerves and in sensory fibres of median and peroneal nerves in patients with obliterative arteriosclerosis. Contrary to the work of Miglietta, their study revealed little change except for some decrease in amplitude of sensory potentials from the median nerve. They stated that, in their patients, the severity of the disease was less than in other series since they had sufficient ischemia in the leg to produce intermittent claudication, but that it was not advanced enough to require amputation.

The studies describing morphological changes in peripheral nerve in patients with peripheral vascular disease have been scarce and incomplete. Gairns and co-workers (1960) found a significant loss of large myelinated fibres in nerves from amputated limbs. Garven et al. (1962), in sections taken at different levels of the posterior tibial axis found a progressive reduction in the number of myelinated nerve fibres and a marked demyelination of them. Chopra and Hurwitz (1967)

studied sural nerve biopsies from six patients with severe peripheral vascular disease, finding variations in internodal length, but no differences in fibre density when compared with control. A similar but more complete study was performed by Eames and Lange (1967), who described the pathological changes in sural nerve biopsies in eight patients with peripheral vascular disease and neurological evidence of neuropathy. They found evidence of segmental demyelination and remyelination, of axonal degeneration and regeneration, and of increase in endoneurial collagen. The unmyelinated fibres were normal, except for some vacuoles and loss of neurofibrils.

Another important study was made by Farinon et al. (1984). They studied 40 patients submitted for amputation of a lower limb. Specimens were also processed for light and electron microscopic study. They found evidence of degeneration and regeneration (myelinated and unmyelinated fibres) and loss of large myelinated fibres.

The present study was performed in order to establish the pathological changes in sural nerves from amputated limbs of patients with arteriosclerosis by using light (including teased nerve fibres) and electron microscopic techniques.

## Materials and methods

The sural nerve from 8 patients suffering from PVD of the lower limbs has been studied. Patients were denominated as PVD 8, PVD 9, PVD 10, PVD 13, PVD 7R, PVD 8R, PVD 9R and PVD 14R.

These patients had an age range from 60 to 80 years. 6 control subjects from a similar age range, denominated as CONTROL 1, 2, 3, 4, 5 and 6 were also studied.

In the PVD patients the biopsy was obtained at the time of amputation of the lower limb affected. The control subjects were brain-dead transplant donors and traumatic amputees.

Patients with other possible causes of peripheral neuropathy, particularly diabetes, carcinoma, collagen disease, alcoholism or neurotoxic drugs and vitamin deficiencies were excluded.

### *Histologic and morphometric procedures*

Light microscopy morphometric observations were made on the sural nerve of PVD patients and age-matched controls. An incision was made one inch behind the lateral malleolus immediately before the leg was amputated. A small piece of sural nerve was gently removed and divided into three portions of 3 mm each. Fixation was undertaken by immersing the specimens in cacodylate-buffered fixatives for three hours at room temperature. After washing with buffer containing 10% sucrose, the specimens were postfixed in 1% cacodylate-buffered osmium tetroxide for three hours, dehydrated in graded concentrations of ethanol, and embedded in EPON with propylene oxide as an intermediary.

For the examination of isolated fibres 1 cm from the

sural nerve was processed in the way already described. Approximately 100 fibres from each patient and controls were obtained by teasing in EPON without accelerator and then mounted on glass slides with EPON.

The isolated nerve fibres were examined for the presence of abnormalities. Only 7 patients were used, and the alterations were classified into 5 categories. Extensive loss of myelin without evidence of remyelination by light microscopy was classified as demyelination. Remyelination was recorded if either single intercalated segments of serial short remyelinated internodal segments were present. Axonal degeneration was recognized by the presence of fibres consisting of rows of osmophilic droplets; axonal regeneration was recorded when internodal length was uniformly and inappropriately short for the diameter of the fibre. Finally, paranodal abnormalities were also recorded in a different category.

Data was represented by mean  $\pm$  SEM.

For light microscopy morphometric observations on the myelinated fibre population, semithin transversal sections 1-1.5  $\mu$ m thick were cut on a Porter Blum MT1 ultramicrotome, stained with thionin and acridine orange and photographed at a final magnification of  $\times 1000$ . All the myelinated fibres in the nerve were counted.

The size-frequency distribution was obtained by measuring fibre diameter using a Sonic digitizer interfaced to a Commodore PET computer which was programmed to classify the diameter into 1.5  $\mu$ m size classes. A systematic random sampling procedure (Mayhew and Sharma, 1984) was employed, and at least 400 fibres in each nerve were assessed.

The fibres sectioned in the paranodal region or at Schmidt-Lanterman incisures were excluded.

Statistical analyses were obtained with the programme STAT and the comparisons were made on mean values ( $\pm$  standard error) by Student's t-test. We have also used ANOVA on analyses followed by the Student-Newman-Kewls test since we observed the existence of two groups of patients in relation to the severity of the disease.

Ultrastructural observations were made on ultrathin sections cut with a Reichert OMU4 Ultracut mycrotome, stained with aqueous uranyl acetate and lead citrate and examined in a Philips EM 201 electron microscope. Ultrastructural observations on myelinated- and unmyelinated-fibre populations, together with an assessment of axon and Schwann cell cytoplasmic inclusions were undertaken.

## Results

### *Teased fiber study*

The results obtained for the human sural nerve in the PVD and controls are shown in Table 1. Less than 16% of abnormalities were encountered in age-matched controls. In comparison with these controls the sural nerve of patients with PVD showed much greater abnormality. The average number of abnormal fibres was  $44.92 \pm 9.55$  with significant difference from controls ( $p < 0.003$ ). The abnormalities consisted of segmental demyelination and remyelination in 5 of the 7 patients.

Wallerian-type axonal degeneration and regeneration were the most important features in the other two patients. Teased fibre preparations from patients with PVD also demonstrated a few fibres in which neither myelin nor osmyophilic droplets could be observed. Such fibres, which probably represented Büngner bands, were not estimated.

#### Morphometric studies

The myelinated fibre density for the ischemic patients and controls are shown in Table 2. There were no significant differences between the means of the two groups. However, three of the patients showed many more alterations than the other five patients when compared to the controls. Due to these differences the patients were classified into two groups. One of them,

severely affected ( $n = 3$ ), and the other one with mild damage ( $n = 5$ ). There were significant differences between the patients with severe and mild neuropathy. Figs. 1 and 2 show semithin sections from one control and one of the severely damaged patients. The loss of myelinated fibres in the ischemic nerve is evident.

The size-frequency distribution (Fig. 3) showed differences between the PVD and the control group. In the PVD patients the bi-modal distribution typical of the controls was not observed. The second peak disappears while the first one, which represents the small diameter fibres, increases.

The size-frequency distribution for the different patients is shown in Figs. 4 and 5. The diversity among the different patients is obvious.

The mean diameter of the pathological fibres did not show significant differences when compared to the controls. However two of the patients were more severely damaged and because of that we analyzed them separately from the other patients (Table 3). There were significant differences between these two patients as compared to the controls and the rest of the patients.

In the study of the myelinated-fibre density, the two patients with significant decrease in the fibre diameter were also included in the severely damaged group.

#### Electron microscopy

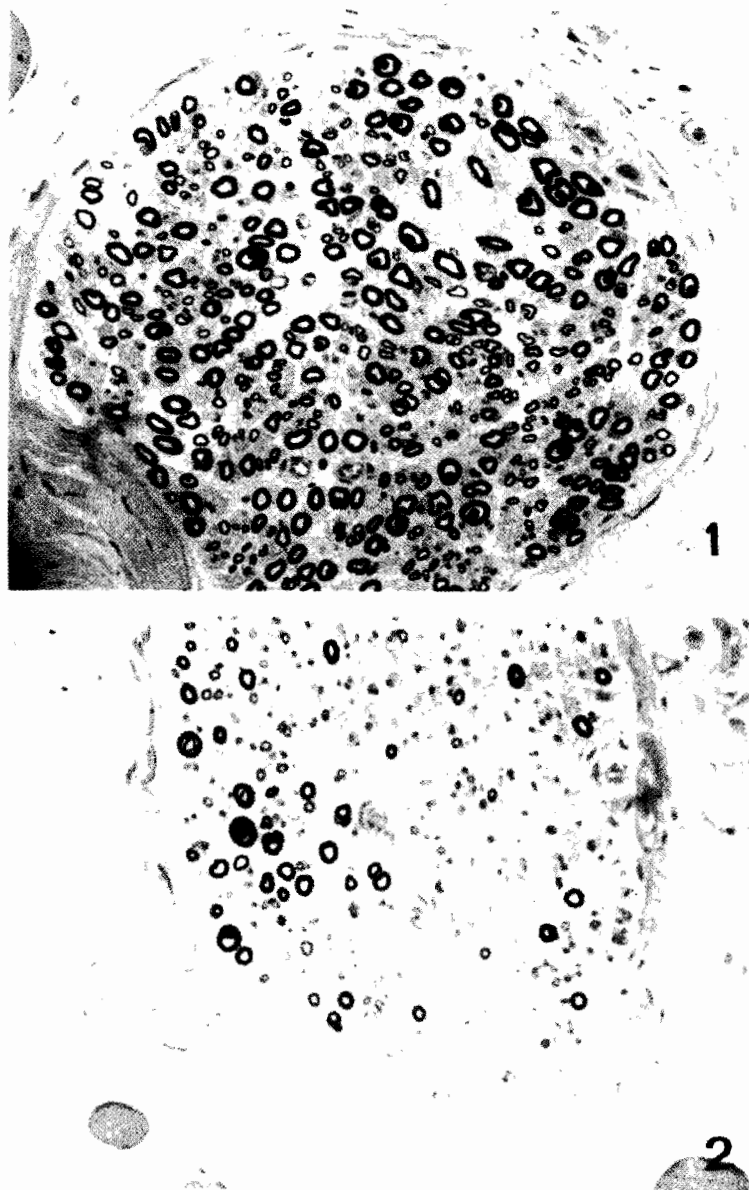
Changes suggesting Wallerian degeneration, regeneration and remyelination were observed in most of the patients.

In three of the patients, few large myelinated fibres were seen. Invasion of the axoplasm by ramifying complexes, and other alterations were seen in myelinated fibres. Some Schwann cells entirely lacked an axon (Figs. 6-9).

Signs of degeneration of unmyelinated fibres: collagen pockets as well as Schwann cell processes surrounded by folded basement membranes; Schwann cell profiles and empty basal lamina were also observed (Figs. 10-12).

In four of the patients, the fibres showed a large amount of Büngner bands and regeneration clusters (Figs. 12-14).

In all patients studied, large nonmyelinated axons as well as other axons which were surrounded by a small myelin sheath were seen, indicating possible signs of remyelination (Fig. 15).



**Fig. 1.** Transverse section of biopsied control sural nerve. Note the abundance of myelinated fibres.  $\times 1,300$

**Fig. 2.** This transverse section shows a reduction in the population of myelinated nerve fibres in a patient with peripheral vascular disease,  $\times 1,300$

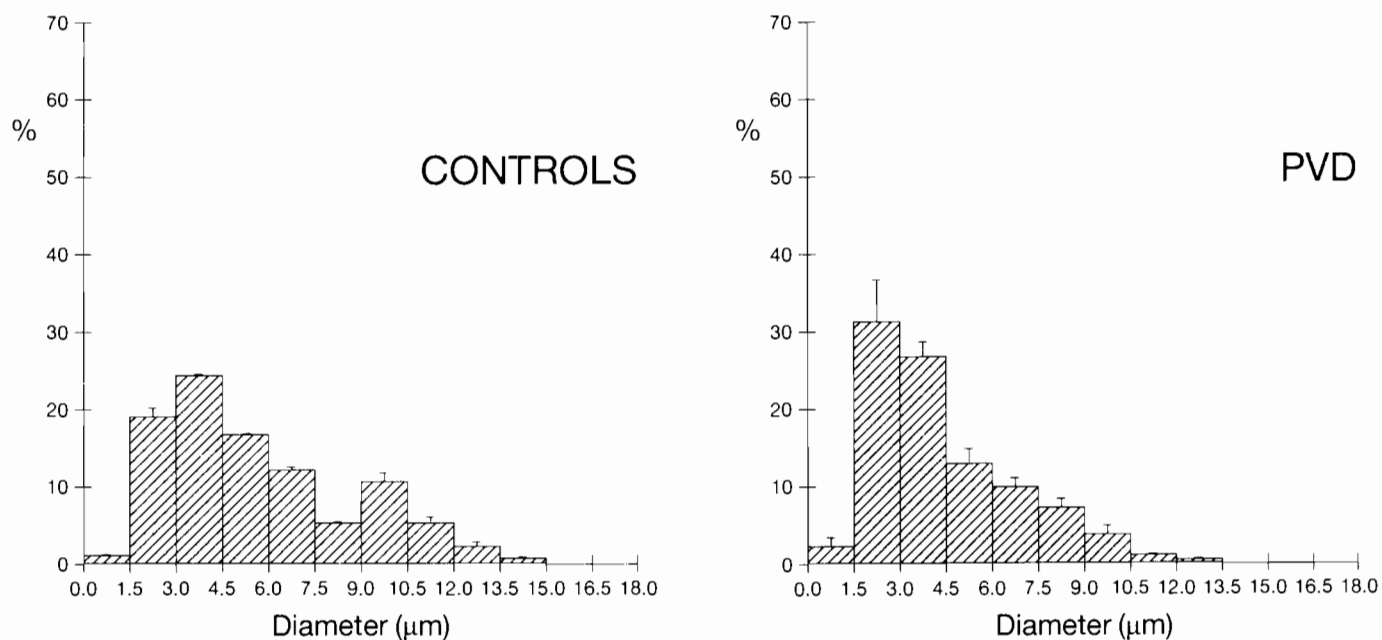
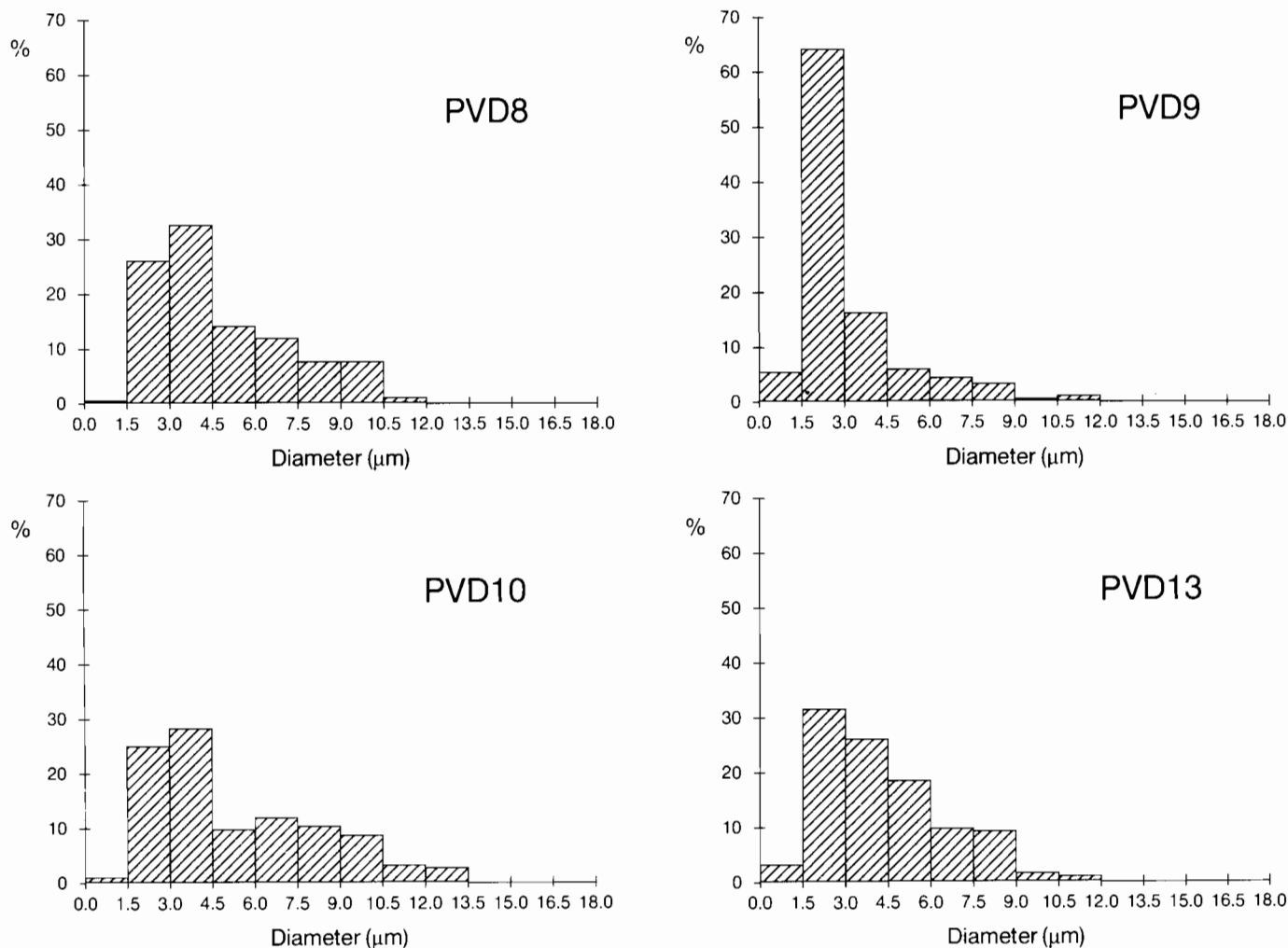
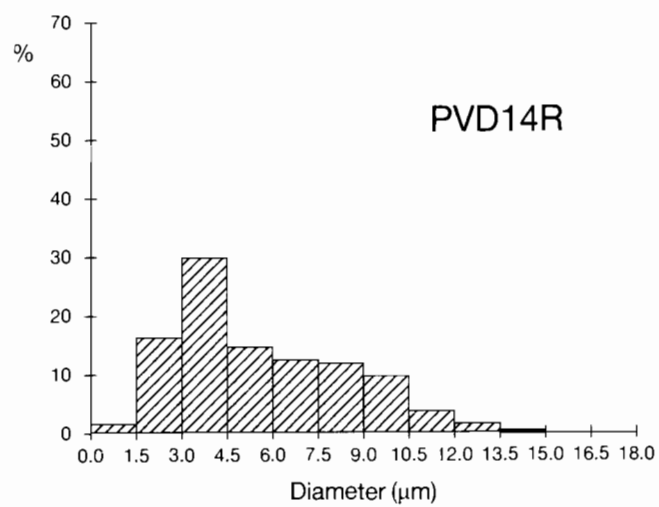
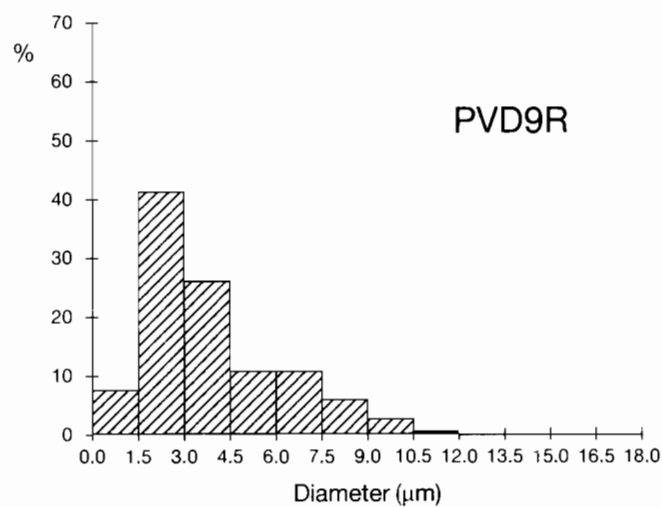
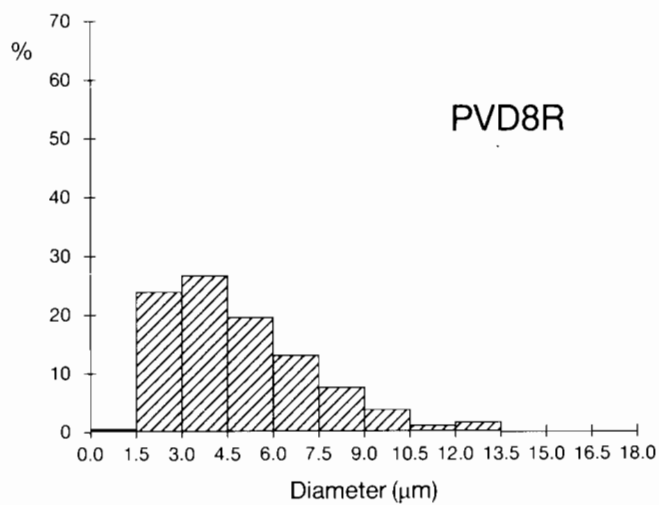
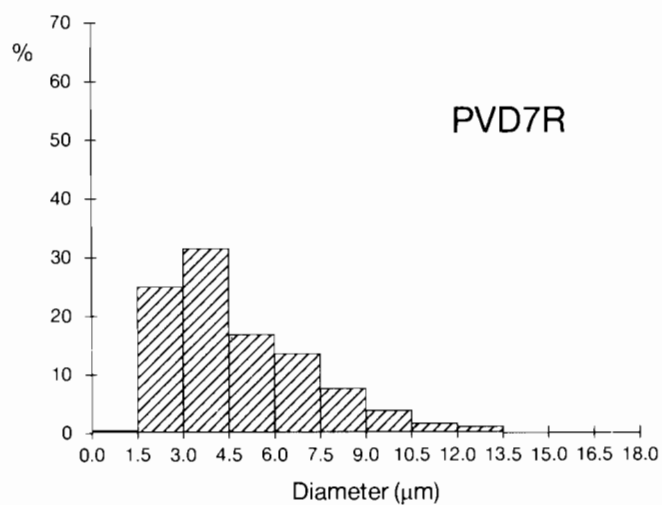


Fig. 3. Composite percentage size-frequency distributions for sural nerve of controls and PVD patients.



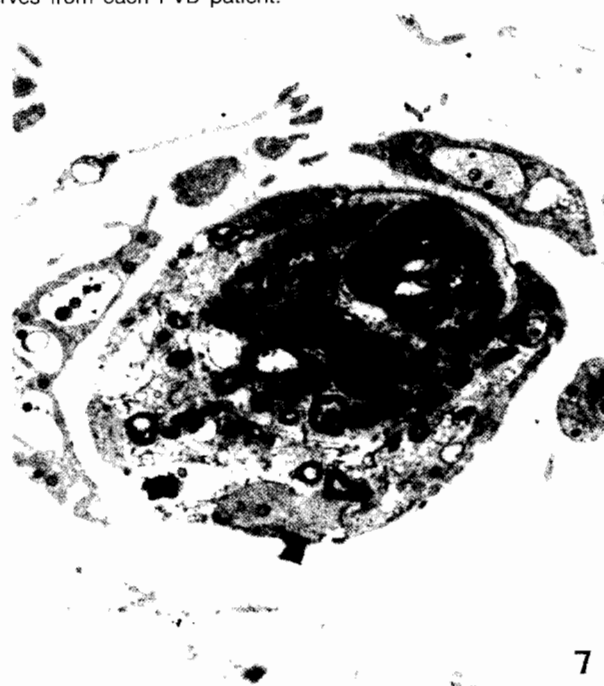
Figs. 4. Size frequency histograms for myelinated fibre from the sural nerves from each PVD patient.



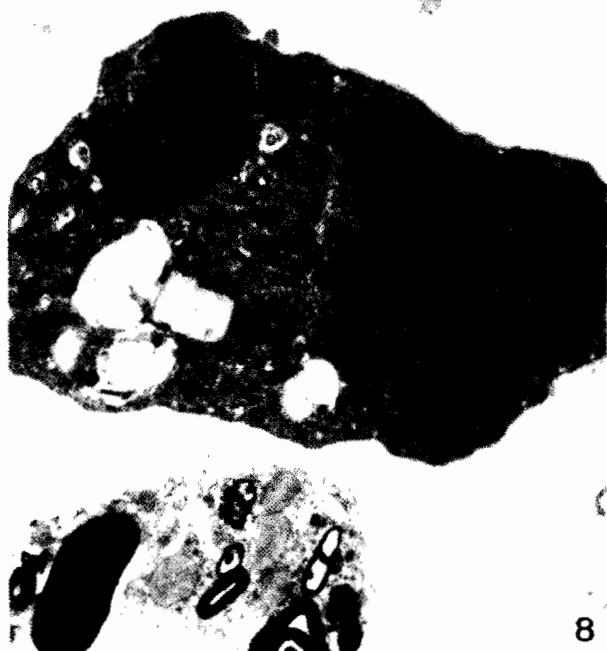
**Fig. 5.** Size frequency histograms for myelinated fibre from the sural nerves from each PVD patient.



**Fig. 6.** Myelinated fibre degeneration. Large myelinated fibre showing extensive honeycombs in the axon network.  $\times 10,100$



**Fig. 7.** Myelin debris from a degenerated myelinated fibre, surrounded by a Schwann cell.  $\times 18,450$



**Fig. 8.** Myelinated fibre undergoing degeneration. Note the myelin debris inside the Schwann cell.  $\times 13,243$



**Fig. 9.** Myelin debris, characteristic of myelinated fibre degeneration.  $\times 15,320$



**Fig. 10.** Note the Schwann cell profiles, signs of unmyelinated fibre degeneration.  $\times 23,240$



**Fig. 11.** Basal lamina from a degenerated fibre.  $\times 20,140$



12



13

**Fig. 12.** Schwann cell profiles and nerve fibres undergoing regeneration, which are surrounded by a single basal lamina (arrows).  $\times 22,130$

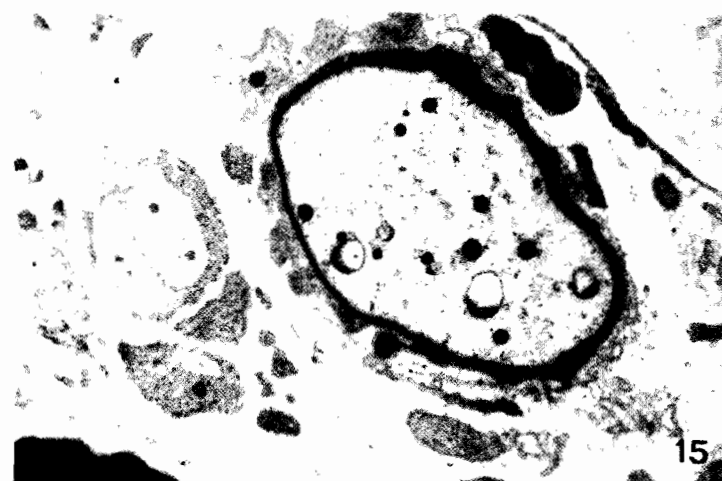
**Fig. 13.** Bands of Büngner showing multiple Schwann cell profiles (arrows). Myelinated fibres undergoing regeneration: note the basal lamina.  $\times 23,122$

**Fig. 14.** Myelinated fibre regeneration: group of myelinated fibres surrounded by a common basement membrane. A regenerative cluster.  $\times 23,100$

**Fig. 15.** Remyelination: Middle-sized axon surrounded by a small myelin sheath.  $\times 7,325$



14



15

## Discussion

This study confirms the observations made by Garven et al. (1962), Eames and Lange (1967) and Farinon et al. (1984), who have claimed a morphological basis for the ischemic neuropathy.

The present results complete these previous reports by using isolated fibre technique, which provides qualitative and quantitative information about axonal and myelin damage, both in morphometric and E.M. studies.

Teased-fibre studies showed an evident sural neuropathy in patients with severe PVD: 44% of abnormal fibres in contrast with 6% in age-matched controls. Two groups of patients were observed. One of them showing mainly axonal degeneration-regeneration, and the other one with demyelination-remyelination. Eames and Lange (1967) reported the same findings, but they only studied two patients and they did not report the frequency of the changes observed. Farinon et al. (1984) also describes these alterations but, without quantifying them or referring to two different types of patients. We think that although the results should be taken with caution,



**Table 1.** Incidence of abnormalities in isolated teased nerve fibres from sural nerves.

	NORMAL FIBRES	AXONAL DEGENER.	AXONAL REGENER.	PARANODAL ABNORMAL	SEGMENTAL DEMYELIN.	SEGMENTAL REMYELIN.	% ABNORMAL FIBERS
CONTROL 1	98	—	—	—	1	2	2.90
CONTROL 2	94	1	3	1	—	1	6.00
CONTROL 3	90	3	3	1	1	2	10.00
CONTROL 4	73	1	2	1	2	7	15.11
CONTROL 5	95	—	—	—	—	4	1.05
CONTROL 6	105	—	—	—	—	1	0.95
PVD 7R	58	—	6	4	13	23	53.84
PVD 8R	72	—	1	4	2	10	10.98
PVD 9R	78	—	3	1	—	22	25.00
PVD 8	47	1	8	—	13	16	37.33
PVD 14R	44	5	12	—	12	24	54.64
PVD 9	16	59	3	—	2	18	84.50
PVD 10	28	32	12	1	5	8	68.11

**Table 2.** Myelinated fibre areas (Mean  $\pm$  SEM) for controls and PVD patients with severe and mild alterations, expressed in  $\mu\text{m}^2$ .

CONTROL n = 6	7.01 $\pm$ 0.57		
MILD n = 5	7.03 $\pm$ 1.52	NS	
SEVERE n = 3	2.67 $\pm$ 1.51	p < 0.05	p < 0.05

**Table 3.** Fibre diameter (Mean  $\pm$  SEM) for controls and PVD patients with severe and mild damage, expressed in  $\mu\text{m}$ .

CONTROL n = 6	4.09 $\pm$ 0.06		
MILD n = 6	4.91 $\pm$ 0.46	—	
SEVERE n = 2	3.36 $\pm$ 0.53	p < 0.05	p < 0.05

our results suggest predominance of Schwann cell damage as contrasted with axonal changes.

Our morphometric findings, compared with the controls of the same age showed important individual variations, with two groups of patients; one of them with mild alterations and the other one severely affected. In the patients with severe damage there was important loss of myelinated fibres. The histograms showed that the sural nerve did not exhibit the two-peaked form. Indeed, the first peak was much more well defined, showing an increase in the number of small fibres. These results have reiterated the observations made by Garven et al. (1962) and Farinon et al. (1984).

The EM observations confirmed the light microscopy findings made by Eames and Lange (1967) and Farinon et al. (1984), with clear evidence of degeneration-regeneration and remyelination. However, we have been unable to demonstrate the onion bulbs described by these authors, even when we have observed large axons surrounded by a thin myelin sheath indicating remyelination.

According to our results, the PVD produces morphological alterations on the peripheral nerve. There are two different manifestations of this disease regarding the pathology of the myelin or the axonal process. In both cases, there are different levels of severity, some of them being more affected than the others.

In conclusion, this study supports the idea that ischaemia is able to cause a structural neuropathy, very similar to that found in Diabetic Neuropathy. Therefore, the fact that ischemia can play a role in the development

of peripheral neuropathy must be borne in mind.

**Acknowledgements.** Carmen Rodríguez-Sánchez and María Medina Sánchez were postdoctoral fellows supported by the University of Oviedo, FICYT and Covadonga Hospital. The authors appreciate the suggestions and critical comments of Dr. A. Menéndez-Pelaez.

## References

- Chopra J.S. and Hurwitz L.J. (1967). Internodal length of sural nerve fibers in chronic occlusive vascular disease. *J. Neurol. Neurosurg. Psychiat.* 30, 207-214.
- Chopra J.S. and Hurwitz L.J. (1968). Femoral nerve conduction in diabetes and chronic occlusive vascular disease. *J. Neurol. Neurosurg. Psychiat.* 31, 28-33.
- Chopra J.S. and Hurwitz L.J. (1969). A comparative study of peripheral nerve conduction in diabetes and nondiabetic chronic occlusive peripheral vascular disease. *Brain* 92, 83-87.
- Chopra J.S. and Hurwitz L.J. (1969). Sural nerve myelinated fiber density and size in diabetics. *J. Neurol. Neurosurg. Psychiat.* 32, 149-153.
- Eames R.A. and Lange L.S. (1967). Clinical and pathological study of ischaemic neuropathy. *J. Neurol. Neurosurg. Psychiat.* 30, 215-226.
- Farinon A.M., Marvini A., Gemignani F., Gobeni E., Bragaglia M.M., Sianesi M. and Tedeschi F. (1984). Skeletal muscle and peripheral nerve changes caused by chronic arterial insufficiency - significance and clinical correlations - histological, histochemical and ultrastructural study. *Clin. Neuropathol.* 5, 240-252.



- Gairns F.W., Garven H.S.D. and Schmidt G. (1960). The digital nerves and the nerve endings in progressive obliterative vascular disease of the leg. *Scot. Med. J.* 5, 382.
- Garven H.S.D., Gairns F.W. and Schmidt G. (1962). The nerve fiber populations of the nerves of the leg in chronic occlusive arterial disease in man. *Scot. Med. J.* 7, 250-265.
- Hutchinson E.C. and Liversedge L.A. (1956). Neuropathy in Peripheral Vascular Disease. *Quart. J. Med., New Series XXV*, 98, 267-274.
- Kumlin T., Seppalainen A. and Railo J. (1963). Electroneurography due to obliterative arteriosclerosis. *Angiology* 25, 373-378.
- Mayhew T.M. and Sharma A.K. (1984). Sampling schemes for estimating nerve fibre size. I. Methods for nerve trunks of mixed fascicularity. *J. Anat.* 139, 45-58.
- Miglietta O. and Lowenthal M. (1962). Nerve conduction velocity and refractory period in peripheral vascular disease. *J. Appl. Physiol.* 17, 837-841.
- Oppenheim H. (1983). Ueber die senile form der multiplen nevritis. *Berl. Klin. Wochenschr.* 30, 589-590.

Accepted July 16, 1990