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A comparison of perineurial and vascular basal laminal changes in diabetic neuropathy

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Abstract Measurements were made of the thickness of the basal lamina of perineurial cells in the sural nerve in a series of patients with diabetic neuropathy and compared with a group of patients with type I hereditary motor and sensory neuropathy (HMSN) and with organ donor control cases. The thickness was significantly greater in the diabetic patients as compared both with the HMSN cases and the organ donor controls. This was most obvious for the intermediate layers of the perineurium. Perineurial basal laminal thickness was only slightly greater in the HMSN cases than in the organ donor controls and the diffference was not statistically significant. The thickening of the perineurial cell basal laminae was compared with the thickening of the basal laminal zone around the endoneurial microvessels. No significant correlation was found either for the diabetic neuropathy or HMSN cases or for the organ donor controls. As had been observed previously, the basal laminal zone around the endoneurial capillaries was of increased thickness both in the diabetic neuropathy and the HMSN cases and, although it was greater for the diabetic neuropathy patients, the difference was not statistically significant. Taken together, these findings indicate that the thickening of the basal lamina of the perineurial cells is a more characteristic feature of diabetic neuropathy than is thickening of the basal laminal zone around the endoneurial capillaries. The results suggest that the causative mechanisms are likely to differ, a conclusion supported by the morphological appearances: the basal laminal thickening around the perineurial cells is uniform, whereas that around the capillaries consists of basal laminal reduplication. Atrophy and necrosis of perineurial cells were observed in patients with diabetic neuropathy but rarely in the cases with HMSN and not in the organ donor cases. This may be similar to the degeneration of endoneurial fibroblasts that has been described as a non-specific finding in neuropathies.

Key words Diabetic neuropathy · Perineurium Basal lamina · Endoneurial capillaries

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Introduction

Thickening of the walls of the endoneurial microvessels was identified as a feature of diabetic neuropathy by Fagerberg [10] and later shown by electron microscopy to be due to reduplication of the basal lamina around the endothelial cells [3, 5, 12]. This change is not specific for diabetes. It occurs with ageing and in other neuropathies [6] but is more frequent in diabetic neuropathy [3, 24, 25]. Bradley et al. [7] showed that, although reduplication occurs around the endoneurial microvessels in type I hereditary motor and sensory neuropathy (HMSN), in comparison with patients with diabetic neuropathy the reduplicated layers of basal lamina tend to be less thick and more often discontinuous. This accords with the finding by King et al. [17] that diabetic Schwann cell basal laminae are more rigid and more resistant to proteolytic degradation than those of non-diabetic Schwann cells.

A further feature of diabetic neuropathy is thickening of the basal laminae that clothe both sides of the perineurial cells. This has been described for the sural nerve [16, 17] and dermal nerves [15] and in dorsal root ganglia [14]. Again, perineurial basal laminal thickening is not specific and occurs with ageing [17].

The mechanisms responsible for the basal laminal changes in the peripheral nerves in diabetes have not been established and it is uncertain whether they are the same for the microvessels and the perineurium. To investigate this we have compared the severity of the basal laminal changes in the perineurium with those in the basement membrane zone surrounding the endoneurial microvessels in a series of patients with diabetic neuropathy. As a disease control, we have again used a series of patients with type I HMSN. In this condition, as already stated, it is known that there is basal laminal reduplication around the endoneurial microvessels, but observations have not previously been made on the perineurial basal laminae.

Some of these results have been published in abstract form [7].

Materials and methods

Case material

The observations were made on 24 patients with diabetes mellitus (11 male, 13 female; mean age 39.2 years, range 23–55 years). All had a distal, approximately symmetric, sensory polyneuropathy. In 17 there was also evidence of autonomic dysfunction, either symptomatically or on formal testing. None had evidence of superimposed focal cranial, thoracoabdominal or limb neuropathies. Of these patients 22 had insulin-dependent, and 2 non-insulin-dependent, diabetes. Absent dorsalis pedis pulses were documented in 4 patients without symptoms of claudication; another had absent distal pulses in both legs with claudication and required a below knee amputation on one side. Neuropathic foot ulceration was present in 5 patients, 2 of which underwent below-knee amputations because of persistent infection. Other clinical details are given by Bradley et al. [6].

Sural nerve fascicular biopsies were obtained from a retromalleolar site under local anaesthetic in 20 patients and a radial nerve biopsy at the wrist in 1 patient, with informed consent and the approval of the Ethics Committee of King's College Hospital. Sural nerves were removed immediately following amputation in 3 cases.

Total sural nerve biopsies were obtained at the same retromalleolar site in 9 organ donor cases (5 male, 4 female; mean age 44.7 years, range 28–55 years) with the consent of the next of kin and the approval of the Ethics Committee at the Royal Free Hospital. All were taken before ventilatory arrest except in 1 instance when the nerve was removed within 45 min of circulatory occlusion. This case was included as histological preservation was excellent. None of these control cases was known to have neuropathy and none had diseases that may give rise to neuropathy.

Retromalleolar sural nerve biopsies from 7 cases of type I HMSN were also studied (5 male, 2 female; mean age 32.1 years, range 11–57 years). The criteria for diagnosis were those of Harding and Thomas [13].

Tissue processing

Portions of the biopsy specimens were fixed in 3% glutaraldehyde in piperazine-*N*-*N'*-bis (2-ethane sulphonic acid) (PIPES) buffer [1] with 2% sucrose. After postfixation in 1% osmium tetroxide in PIPES buffer containing 1.5% potassium ferricyanide, the specimens were dehydrated through ascending concentrations of ethanol and embedded in Araldite or Durcupan via 1,2-epoxypropane. Transverse semithin sections were stained with thionin and acridine orange [22] for orientation. Transverse ultrathin sections were contrasted with uranyl acetate and lead citrate and examined in a JEOL 100CX electron microscope.

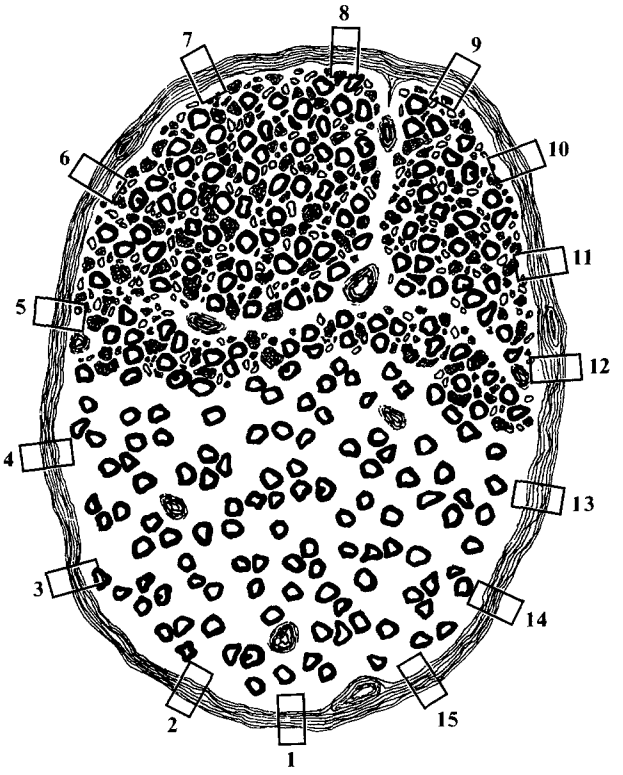

Fig. 1 Diagram of transverse section through a fascicle of the sural nerve showing the situation of the 15 photographic montages obtained for mensuration of perineurial basal laminal thickness

Data collection

From the transverse ultrathin sections of the nerve biopsy specimens viewed by electron microscopy, 15 sites were chosen at random around the perimeter of the fascicle (Fig. 1) and the full thickness of the perineurium photographed at a magnification of × 8,300. Invariably more than one micrograph was needed to span the full thickness of the perineurium. A montage was constructed from the resulting micrographs.

Preliminary observations suggested that the thickness of the perineurial basal lamina varies in a systematic way across the width of the perineurium. The perineurial cell layers were, therefore, numbered from 1 to 10, beginning from the epineurial side. Where there were less than 10 layers, for example 8, the external and internal layers were designated 1, 2 and 3 and 8, 9 and 10, respectively and the 2 remaining central layers were labelled 5 and 6.

For each montage 18 pencilled lines, 1 cm apart, were drawn perpendicular to the perineurial cell layers, crossing the entire width of the perineurium. These lines were divided into two sets of 9 and the lines numbered 1–9 consecutively in each set. A table of random numbers was then used to select ten numbers and the correspondingly numbered pencilled lines were overdrawn in ink (Fig. 2). Direct computer measurements were made, using a digitising tablet and pen, of the thickness of the basal lamina on both the internal and external aspects of each numbered perineurial cell layer (Fig. 2). Median basal laminal thicknesses were obtained from the observations made along all 10 perpendicular lines.

The observations on the thickness of the basal laminal zone of the endoneurial microvessels were those obtained by Bradley et al. [6]. All the microvessels were classifiable as capillaries on the criteria of Bell and Weddell [4]. The measurements of the thickness of the basal laminal zone were derived from measurements of the area, measured on electron micrographs, of the zone enclosed by the outer perimeter of the endothelial cells and the outermost layer of basal lamina.

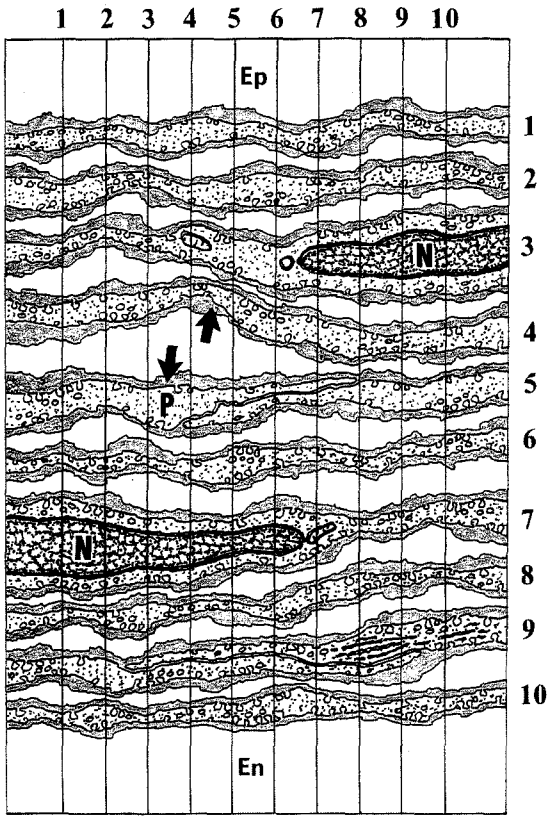

Fig. 2 Diagram of one of the 15 photographic montages shown in Fig. 1 indicating the 10 randomly selected vertical lines along which the thickness of the perineurial basal laminae were measured. The lines are drawn with equidistant spacing for illustrative purposes (*Ep* epineurium, *En* endoneurium, *N* nuclei of perineurial cells, *P* perineurial cell, *arrows* perineurial basal laminae)

Statistical analysis

Statistical comparisons were performed by Student's *t*-test or, if the data were not normally distributed, the Kolmogorov Smirnov test.

Results

Qualitative observations

The normal appearances of the perineurium are shown in Fig. 3. The basal laminal thickening in the perineurium in the present series of cases of diabetic neuropathy was usually uniform (Fig. 4) or at times irregular giving rise to a 'moth-eaten' appearance (Fig. 5). Reduplication of the basal lamina was not observed. Cellular debris and calcified deposits were often observed between the perineurial cell layers. This was uncommon in the HMSN and organ donor cases.

In the diabetic patients evidence of degenerative changes in the perineurial cells was not infrequent. This sometimes took the form of atrophy, so that a narrow perineurial cell with a substantial gap between the basal lamina on either side was seen (Fig. 4). At other times, disintegration or loss of perineurial cells was observed (Fig. 5), resulting in the presence of cell debris between adjacent basal laminae or of paired basal laminae with no intervening perineurial cell. Such appearances were very rare in the HMSN cases and were not encountered in the organ donor control cases.

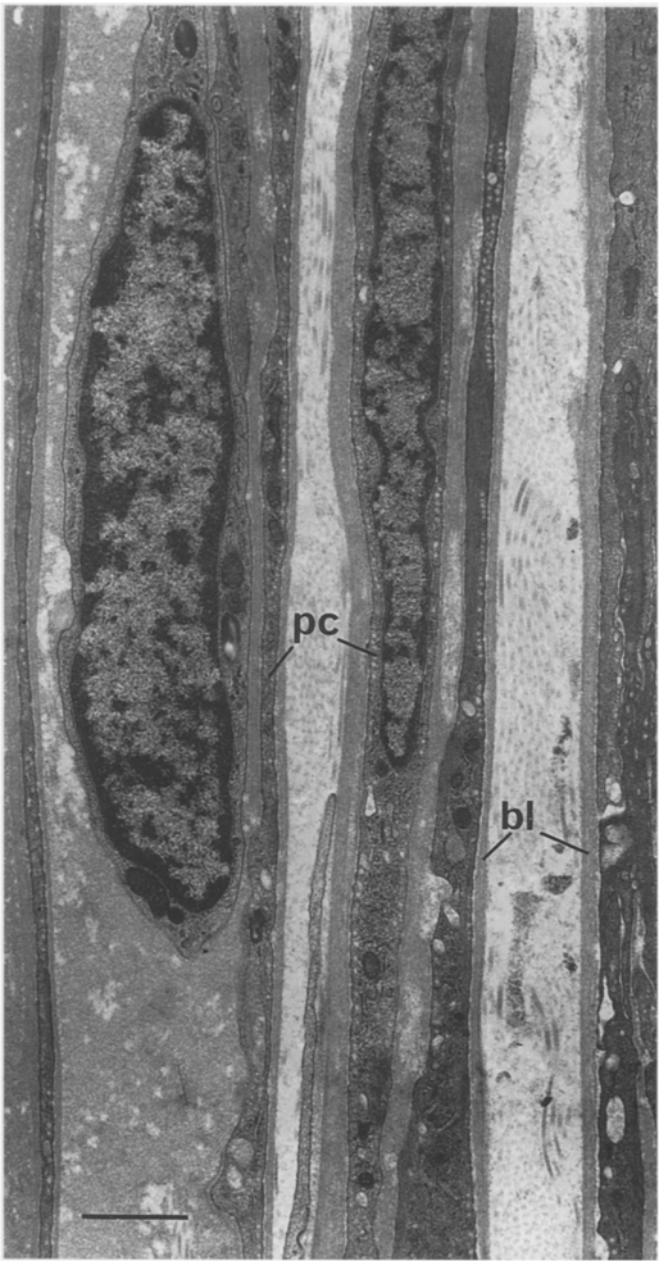

Fig. 3 Electron micrograph of transverse section through the perineurium from the sural nerve of an organ donor control subject. The perineurial cells (*pc*) are covered both on their internal and external aspects by basal lamina (*bl*) and are separated by zones composed of collagenous connective tissue. An additional cell of uncertain nature is present within the interlamellar space on the left. *Bar*=1 μm

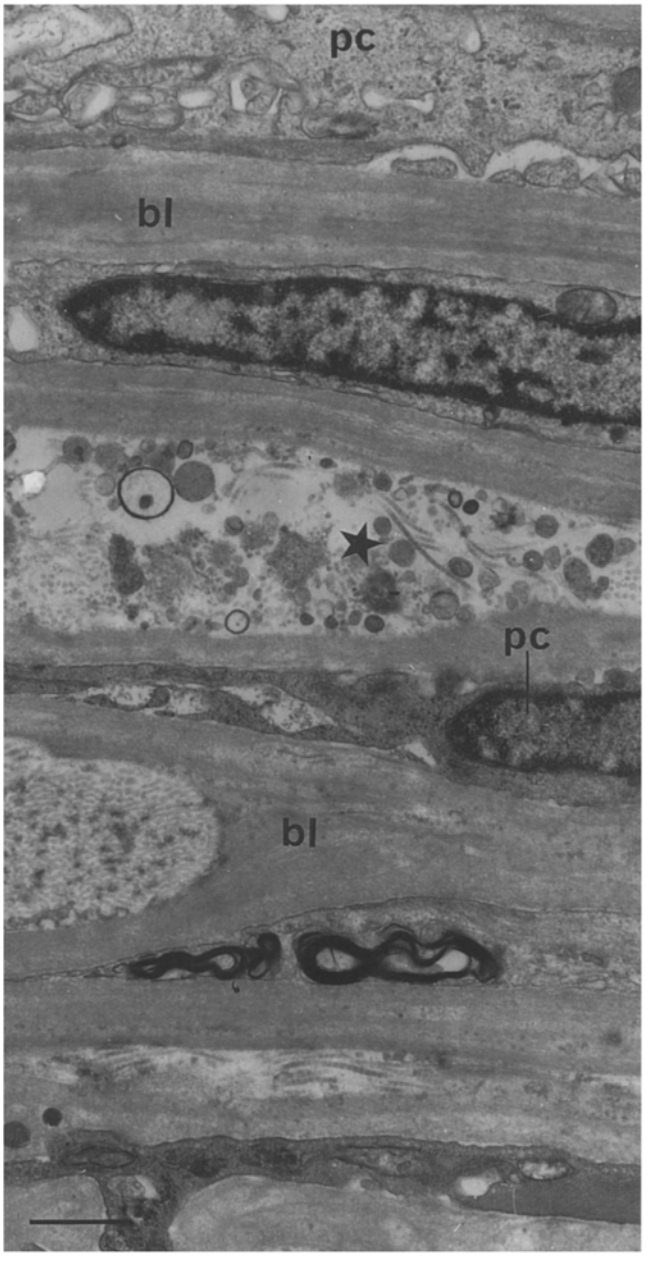

Fig. 4 Electron micrograph of transverse section through perineurium from the sural nerve of a patient with diabetic neuropathy showing greatly thickened basal laminae (*bl*). One interlamellar space (*star*) contains a considerable amount of cell debris (*pc* perineurial cells) *Bar*=1 μm

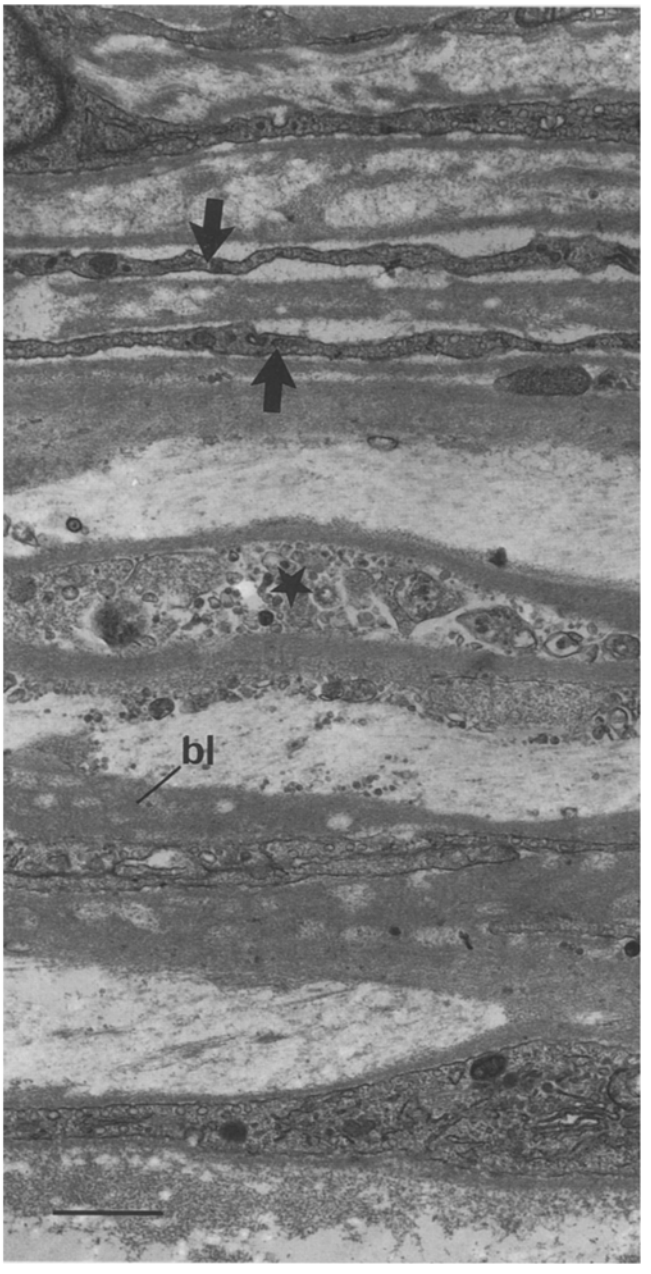

Fig. 5 Electron micrograph of transverse section through perineurium from sural nerve of a patient with diabetic neuropathy showing thickened 'moth-eaten' basal lamina (*bl*) and a disintegrating degenerate perineurial cell (*star*). Atrophic perineurial cells (*arrowed*) are visible to the top of the figure. *Bar*=1 μm

Quantitative observations

The median thickness of the perineurial basal laminae showed no significant increase with age in the organ donor control cases ($r = 0.0662$), but the age range was limited (28–55 years) and no elderly individuals were included. There was a significant increase with age both in the diabetic ($r = 0.4874$, $P < 0.01$) and HMSN ($r = 0.7605$, $P < 0.05$) nerves, possibly related to disease duration.

The median thickness of the basal laminae was consistently and significantly greater ($P < 0.01$) in the diabetic patients for all perineurial layers than the organ donor control cases. This was most obvious for the central layers (Fig. 6). The thickness was also greater in the HMSN cases, although the differences were less pronounced for the inner perineurial layers (Fig. 6). The basal laminae for the HMSN cases were significantly thicker than those of the organ donor cases for only some of the layers, this again being most obvious for

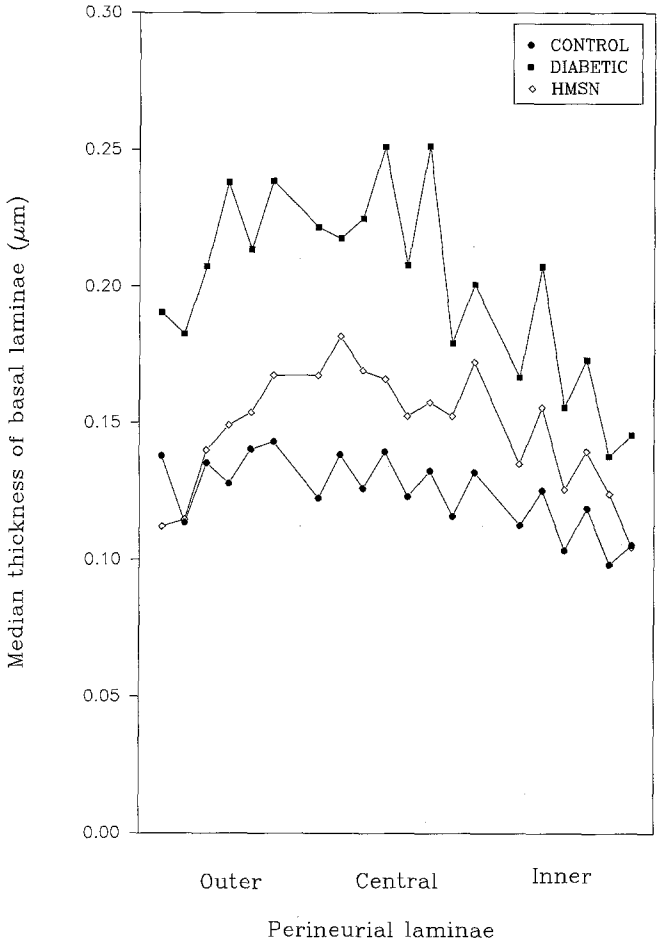

Fig. 6 Variation in median thickness of basal laminae across the thickness of the perineurium for organ donor control subjects (*filled circles*) and for patients with diabetic neuropathy (*filled squares*) and hereditary motor and sensory neuropathy (HMSN) (*open diamonds*)

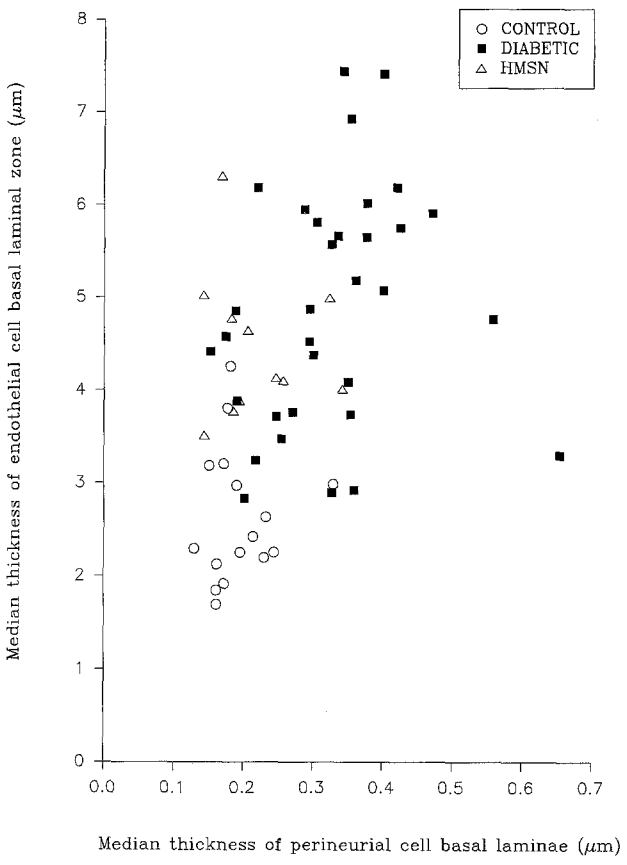

Fig. 7 Plot of median thickness of basal laminal zone surrounding endoneurial capillaries against median basal laminal thickness for organ donor control subjects (*open circles*) and patients with diabetes (*filled squares*) and HMSN (*open diamonds*)

the intermediate layers. When the data for all ten layers were pooled, there was no significant difference.

The median thickness of the perineurial basal laminae has been plotted against thickness of the basal laminal zone of the endoneurial capillaries in Fig. 7. This shows the increase in the thickness of the capillary basal laminal zone in both diabetic and HMSN cases but emphasizes the greater degree of thickening in the diabetic patients, particularly for perineurial thickness. When both the median and maximum values for perineurial and capillary basal laminal thickness were compared, no significant correlation was observed for the organ donor control, diabetic or HMSN cases (Table 1).

Discussion

The lamellated perineurium is made up of concentric layers of cells which are derived from fibroblasts [9, 23]. Each layer is bounded on both internal and external aspects by basal lamina. The present study has confirmed previous observations [14–17] that it is thickened in diabetic neuropathy and has shown that this may be a more characteristic feature of diabetes than the reduplication of basal lamina around endoneurial microvessels [3, 5, 12, 25]. In comparison with patients with HMSN, in our previous study we found that the thickness of the basal laminal zone of endoneurial capillaries was somewhat greater in diabetic neuropathy but not significantly so. In the present observations, the thickness of the perineurial basal laminae was significantly

Table 1 Correlation between median and maximum perineurial basal laminal thickness and the thickness of the basal laminal zone of endoneurial capillaries for the organ donor, diabetic neuropathy and HMSN cases (*HMSN* hereditary motor and sensory neuropathy, *n* number of fascicles examined, *n.s.* not significant)

	n	r	P
Organ donor controls			
Median	16	0.0806	n.s.
Maximum	16	0.0905	n.s.
Diabetic neuropathy			
Median	34	0.3604	n.s.
Maximum	34	0.3161	n.s.
HMSN			
Median	10	0.1256	n.s.
Maximum	10	0.0002	n.s.

greater than in both the HMSN cases and the organ donor control nerves. The increase in the HMSN cases, in comparison with the organ donor cases, was not statistically significant.

The present results suggest that the mechanisms leading to the thickening of the perineurial basal laminae and the endoneurial capillary basal laminal zone are likely to differ. The morphological appearances are dissimilar in that the perineurial basal laminal thickening is uniform, whereas around endoneurial capillaries it consists of reduplication. Moreover, there is no correlation between the severity of the basal laminal thickening in the perineurium and that around endoneurial microvessels.

The reasons for these basal laminal changes are obscure. It seems possible that, for the endoneurial capillaries, they may be related to endothelial cell hyperplasia, the basal lamina being 'shed' by the endothelial cells following cell division. Alternatively, it might reflect recurrent changes in the calibre of the endoneurial microvessels [6]. Such explanations are not likely to apply to the perineurium as the thickening is not a reduplication, despite the fact that perineurial cell degeneration or necrosis was observed.

Basal laminae are composed of collagens and glycoproteins, the main glycoprotein being laminin. Fibronectin is another important component. In the perineurium, the collagens are mainly types IV and V [21]. Little is known concerning the specific changes in perineurial basal laminae in diabetes but, in general, the main feature appears to be an accumulation of over-glycosylated collagens with a very slow turnover. For the renal glomerular basal lamina, abnormal glycosylation is known to reduce its susceptibility to proteolytic digestion [18]. Reduced turnover of perineurial basal lamina could, therefore, be an explanation for its thickening. Alternatively, or additionally, increased synthesis could be involved. Increased renal glomerular basement membrane synthesis has been found in alloxan-diabetic rats [8], and hyperglycaemia due to insulin deficiency has been shown to lead to accelerated basal laminal protein synthesis in EHS (Engelbreth-Holm-Swarm) tumours in vitro [20]. Comparison of perineurial basal laminae in dermal nerves by Beggs et al. [2] between monozygotic twins discordant for diabetes mellitus indicated that dysmetabolism and not genetic factors is responsible. It is also of interest that these authors found a significant reduction in dermal and sural nerve perineurial basal laminal thickness 2 years after isogenic pancreatic transplantation with euglycaemia in the diabetic twin.

Why the perineurium should be affected in particular in diabetes is uncertain. Schwann cell basal laminae are not thickened, although they exhibit other changes [17]. Variable changes are also observed in other tissues. Murrah et al. [19] found that renal tubule and gingival capillary basal laminae become thickened in diabetes, whereas basal laminae around parotid duct and acinar glands do not.

The necrotic changes in the perineurial cells observed in the present study call for comment. Grehl and Schröder [11] have documented the degeneration of endoneurial fibroblasts in a variety of neuropathies, comprising polyarteritis nodosa, HMSN, paraproteinaemic neuropathy and chronic inflammatory demyelinating polyneuropathy. Diabetic neuropathy was not included. The degeneration of endoneurial fibroblasts was considered to be a non-specific sign of peripheral neuropathy, although amongst those examined it was most evident in polyarteritis nodosa. In view of the derivation of perineurial cells from fibroblasts [9, 23], it is possible that the degeneration and loss of perineurial cells observed in the present study represents a similar phenomenon. It will be of interest to establish whether endoneurial fibroblasts are also affected in diabetic neuropathy.

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