J Clin Pathol 1985;**38**:1030–1038

Small vessel disease in progressive diabetic neuropathy associated with good metabolic control

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SUMMARY Clinical, electrophysiological, and electron microscopical data are presented on 10 diabetic patients with severe progressive neuropathy, predominantly motor in type, in the presence of good blood glucose control, and for one patient with painful neuropathy and third cranial nerve palsy. Endothelial cell hyperplasia was seen in small vessels in all cases, and seven patients showed plugging of the vascular lumen by degenerate cellular material and electron dense protein. It is suggested that these cells desquamate and occlude smaller peripheral vessels at a point of narrowing. In one case the lumen of a vessel was occluded by thrombus. Electron microscopical examination showed a vessel occluded by degranulated platelets.

Electrophysiological studies showed a pattern of denervation that was asymmetrical and distally predominant in some patients, suggesting that the neuropathy, at least in part, relates to multiple small infarcts.

Peripheral nerves in diabetes sustain widespread damage consisting of segmental demyelination[1] and axonal degeneration.[2] Various clinical syndromes occur as a result, and it is now customary to refer to "diabetic neuropathies" rather than to the single entity of diabetic neuropathy. The aetiology of diabetic neuropathy is still under question, and the relative importance of metabolic and vascular factors is uncertain.

Many of the syndromes of diabetic neuropathies manifest diffuse sensory or motor features, suggesting a metabolic effect; it is possible, however, that diffuse small vessel disease could also result in symmetrical abnormalities of nerve function. In 1959 Fagerberg described "vascular lesions in peripheral nerve in the form of hyalinisation, caliber reduction and thickening of the wall which were more common in patients with diabetic neuropathy than in other groups."[3] He attributed such change to progressive degenerative disease and assumed that they were responsible for the structural damage to the myelin sheath in diabetic patients. Timperley *et al* described plugging of small blood vessels with fibrin or thrombus, and also focal areas of necrosis and loss of nerve fibre.[4] Similarly, Williams *et al,* in an electron microscopical study, described thrombi and hyperplasia of vascular endothelial cells sufficient to occlude the lumen of small vessels.[5] They suggested that hyperplasia of endothelial cells could result in profound alterations of local blood flow and might account for a symmetrical form of neuropathy.

Accepted for publication 15 May 1985

Material and methods

We studied 11 patients with diabetes (Table 1). Nine of these had type II diabetes and two type I. The age range of the patients was 31–77 (mean 58·2) years with a duration of diabetes of one to 25 (mean 6·2) years. The duration of the neuropathic syndrome was relatively short at two to 24 (mean 12) months. All but one patient had a range of random blood glucose concentrations consistently below 9 mmol/l (162 mg/100 ml). The glycosylated haemoglobin values in the patients were near to the normal range (mean 46·3 mmol HMF/mol haemoglobin; normal range 29–39 mmol HMF/mol haemoglobin).

CLINICAL FEATURES OF THE NEUROPATHIC SYNDROME

The striking feature of these patients was the profound motor disability; all of them had severe wasting of muscles and predominantly incapacitating weakness, which in some cases exclusively affected the proximal leg muscles. In all patients the progress and development to severe muscle weakness varied from two to six months. The neuropathy seemed to be progressive, for there was no evidence of

improvement in any patient. Four of the patients were subsequently virtually confined to wheelchairs.

Sensory symptoms, if present, were minimal and consisted of trivial intermittent tingling or numbness of the feet and, rarely, the hands. Two patients had no sensory symptoms or signs. In the others some patchy superficial sensory blunting in the feet and fingers was evident. The reflexes were uniformly absent in the legs and depressed or absent in the arms.

A biothesiometer showed considerable impairment of vibration perception threshold. The mean value for these patients was 22·5 units (range 10–46). This compares with a normal range on the big toe of two to 10. One patient with painful neuropathy developed third cranial nerve palsy, although well controlled with an insulin infusion pump.

Although the ideal control group would have been diabetic patients without neuropathy, we thought that it would be unethical to biopsy these patients.

CONTROL SUBJECTS

Six patients with severe peripheral neuropathy unassociated with diabetes were studied as a control group (Table 2). Their ages ranged from 45 to 69 (mean 58) years. The duration of the neuropathy ranged from four months to five years. The patient in whom the duration of neuropathy was shortest had carcinoma of the lung; the others had chronic motor neuropathy, chronic sensory motor neuropathy, the Roussy Levy syndrome, autoimmune neuropathy, and mononeuritis multiplex in which there was no evidence of collagen vascular disease. In all these patients there was a severe degree (Medical Research Council grade 3) of weakness of both arms and legs, proximally and distally, and in three patients sensory symptoms were present to a mild degree. Random blood glucose estimations and glycosylated haemoglobin concentrations in all these patients refuted the diagnosis of diabetes.

Six other cases were studied. These were all traumatic deaths, and a necropsy specimen of sural nerve was obtained as soon as possible after death. This group acted as a control in which there was no known evidence of peripheral nerve disease. The ages of these patients were 72, 71, 63, 58, 18, and 17 years.

Histology

Under local anaesthesia the sural nerve was exposed behind the lateral malleolus. After removal small fragments of nerve were fixed in 3% glutaraldehyde in 0·1M phosphate buffer (pH 7·35) for one hour at room temperature with constant agitation. The pieces of nerve were then carefully trimmed to remove excess connective tissue and fat and were fixed for a further hour in fresh glutaraldehyde. They were then washed several times in 0·1M phosphate buffer (pH 7·35) and postfixed in 1% osmium tetroxide at room temperature for one hour with constant agitation. The nerve was then washed in phosphate buffer and finally rinsed in distilled water. After dehydration the tissue was embedded in epoxy resin. About 2 mm of nerve was fixed in 10% neutral formalin and 3 mm was fixed in 1% buffered osmium tetroxide for teased nerve fibre preparations. Nerve embedded in paraffin was sectioned and stained with haematoxylin and eosin, solochrome cyanine for myelin, Marcius scarlet blue, and Masson's trichrome stain.

Neurophysiology

A standard system was used (Medelec, type MS6). In all patients, whether controls or those with diabetes, electromyography was carried out on the deltoid, abductor pollicis brevis, vastus medialis, and extensor digitorum brevis muscles, and conduction studies of the median, peroneal, and sural nerves were also carried out. The conduction studies com-

Table 1 *Clinical features of diabetic patients*

Age (years)	*Duration of:*		*Type of diabetes*	*Weakness in arm**		*Weakness in leg**		*Sensory signs†*		*Retinal disease*	*Renal disease*	*Haemoglobin A*	*Treatment*
	Neuropathy (months)	*Diabetes (years)*		*Proximal*	*Distal*	*Proximal*	*Distal*	*Arms*	*Legs*				
56	12	18	I	3	4	1	4	Absent	+	Present	Absent	42	Insulin
77	18	6	II	4	5	0	3	Absent	+	Absent	Absent		Oral drugs
42	4	2	II	5	5	4	5	Absent	+	Present	Absent	49	Oral drugs
66	8	7	II	4	4	Right 2 Left 3	Right 1 Left 4	Absent	+	Absent	Absent	60	Oral drugs
31	2	1	I	3	5	3	5	+	++	Absent	Absent	47	Insulin
63	12	1	II	4	4	3	2	Absent	+	Absent	Absent	37	Oral drugs
54	12	25	II	4	5	3	5	Absent	Absent	Absent	Absent	45	Insulin
61	24	2	II	4	5	4	5	+	+	Absent	Absent	48	Insulin
58	24	2	II	4	5	3	4	Absent	Absent	Absent	Absent	45	Diet
74	5	5	II	5	3	4	1	+	+	Absent	Absent	42	Oral drugs

*Motor function assessed according to Medical Research Council grading of muscle power.
†++ = Moderate impairment, + = mild impairment.

prised an asessment of: in the median nerve, motor conduction in fibres to abductor pollicis brevis and orthodromic sensory conduction between the second digit and wrist; in the peroneal nerve, motor conduction in fibres to extensor digitorum brevis; and in the sural nerve, orthodromic conduction between lateral malleolus and calf. A conventional concentric needle electrode (Disa, type 13L49) was used for electromyography and to record evoked muscle action potentials. In all other cases standard surface electrodes were used (Medelec, types E/DPNS, E/DS-K, EL210M).

Results

Electromyographic evidence of denervation was graded as follows: complete (++++), no motor units under voluntary activity could be recorded; severe (+++), a pattern of discrete activity at maximal voluntary effort. This was usually associated with motor units of abnormally long duration; moderate (++) and mild (+), corresponding to the degree of reduction in the pattern of activity. To avoid the erroneous classification of normal muscle activated submaximally, denervation was diagnosed in these instances only when associated with motor units of abnormally long duration, indicative of regeneration of motor nerves.

In the group with diabetes all patients also showed evidence of motor neuropathy, which, in seven cases, was moderate or severe. The changes tended to be more pronounced distally than proximally and more severe in the leg than the arm. In two patients, however, there was disproportionately severe proximal denervation, and in one patient denervation was greater in the arm than in the leg. Severe sensory denervation was present in all but one patient (Table 3). Although some slowing of nerve conduction was found in most patients in this group, it was often modest and probably attributable to degeneration of the fastest conducting fibres.

The control group of patients with non-diabetic neuropathy showed evidence on electromyography of severe motor neuropathy in all cases and, with one exception, severe sensory denervation. Pronounced slowing of nerve conduction velocity, implying demyelination, was found in two patients; otherwise, the appearances were those of a predominantly axonal form of neuropathy (Table 4). The overall degree of abnormality was, if anything, more pronounced in the control series with non-diabetic neuropathy than in the diabetic group.

Evidence of demyelination was seen in teased nerve fibre preparations from all diabetics. Electron microscopical examination showed evidence of axonal degeneration in all cases, which was often severe.

Electron microscopical examination showed hyperplasia of endothelial cells in some small endoneurial blood vessels in all diabetics examined (Fig. 1). A vessel with hyperplastic endothelial cells was defined as a vessel in which the lumen was occupied by these cells to the extent that the luminal borders were, in places, in contact with one another. Some vessels showing hyperplasia of endothelial cells also showed spearation of adjacent cell borders extending down to and affecting the base of the cells, opening up possible channels of communication between the lumen of the vessel and the vessel wall (Fig. 2). In longitudinal sections endothelial cells sometimes extended well into and along the lumen of the vessel (Fig. 3).

Seven cases showed plugging of the lumen of small vessels with cellular material; the cells plugging the lumen of these vessels were degenerate with appreciably pyknotic nuclei (Fig. 4), and in some cases the cells were disintegrating, releasing cytoplasmic debris into the vessel lumen. These plugs of degenerate cells were frequently associated with electron dense protein material, which was probably, at least in part, fibrin. In one case the lumen of a vessel was occluded by thrombus and fibrin was seen to be tracking through the vessel wall in sections studied by light microscopy (Fig. 5); electron microscopical examination showed a vessel containing a plug of degranulated platelets (Fig. 6), and in

Table 2 *Clinical features of control patients with neuropathy*

Age (years)	*Diagnosis*	*Duration of disease*	*Weakness in arm**		*Weakness in leg**		*Sensory signs†*	
			Proximal	*Distal*	*Proximal*	*Distal*	*Arms*	*Legs*
62	Autoimmune	5 years	4	4	4	3	+	+
69	Cancer of lung	4 months	5	4	3	4	Absent	+
60	Roussy-Levy	3 years	3	3	3	3	Absent	Absent
50	Chronic motor	2 years	3	4	3	3	Absent	Absent
64	Chronic sensorimotor	1 year	5	4	3	2	+	+
45	Mononeuritis multiplex	1 year	Moderately severe bilateral median nerve palsies Slight bilateral ulnar nerve palsies					

*Motor function assessed according to Medical Research Council grading of muscle power.
†+ = mild impairment.

Table 3 *Results of electromyography and nerve conduction studies in diabetic patients*

Deltoid	*Abductor pollicis brevis*	*Vastus medialis*	*Extensor digitorum brevis*	*SCV (m/s)*	*Median*			*Peroneal*		*Sural*	
					SAP (μV)	*MCV (m/s)*	*DL (ms)*	*MCV (m/s)*	*DL (ms)*	*SCV (ms)*	*SAP (μV)*
+	+	+	++++	56	4	51	4·3	36	5·8	26	2
+++	+	+++	++++	34	1	59	5·6	—	—	—	—
Normal	+	Normal	+++	40	1	48	6·3	41	5·2	34	1
+	++	+	++++	43	2	47	4·4	—	—	29	1
+	+	+	+	44	4	48	4·4	32	6·5	—	—
+	++	++	++++	41	1	38	4·4	—	—	—	—
+	++	+	+	53	9	56	3·7	40	5·0	37	9
+	+	Normal	+++	45	2	44	3·6	39	5·1	45	1
+	Normal	Normal	+	58	4	54	4·3	35	5·8	—	—
Normal	+	+++	++++	38	1	45	3·8	28	6·7	—	—
Normal ranges:				46–71	5–25	48–71	2·5–4·4	40–59	3·0–6·2	38–63	3–24

Electromyographic evidence of denervation: ++++ = complete, +++ = severe, ++ = moderate, + = mild.

Table 4 *Results of electromyography and nerve conduction studies in control patients with neuropathy*

Deltoid	*Abductor pollicis brevis*	*Vastus medialis*	*Extensor digitorum brevis*	*SCV (m/s)*	*Median*			*Peroneal*		*Sural*	
					SAP (μV)	*MCV (m/s)*	*DL (ms)*	*MCV (m/s)*	*DL (ms)*	*SCV (ms)*	*SAP (μV)*
+++	+++	+++	++++	—	—	15	14·5	—	—	—	—
++	+++	++++	+++	58	4	55	4·0	35	7·1	—	—
+	+++	++	+++	—	—	28	63·0	—	—	—	—
+++	+	+++	+++	58	6	50	4·2	44	4·8	48	7
Normal	++	++	++++	54	1	57	4·0	—	—	—	—
++	+++	+	++++	—	—	34	4·6	32	5·1	35	1
Normal ranges:				46–7	5–25	48–71	2·5–4·4	40–59	3·0–6·2	38–63	3–24

Electromyographic evidence of denervation: ++++ = complete, +++ = severe, ++ = moderate, + = mild.

SCV = Sensory conduction velocity; SAP = sensory action potential; MCV = motor conduction velocity; DL = distal latency.

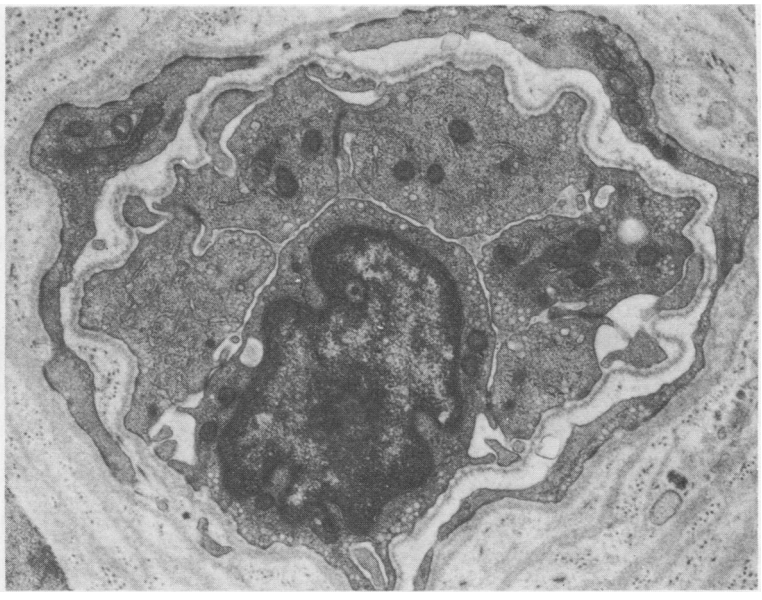

Fig. 1 *Electron micrograph showing hyperplasia of endothelial cells in small blood vessel resulting in almost total occlusion of lumen. Note also pronounced thickening of vessel wall. ×7500.*

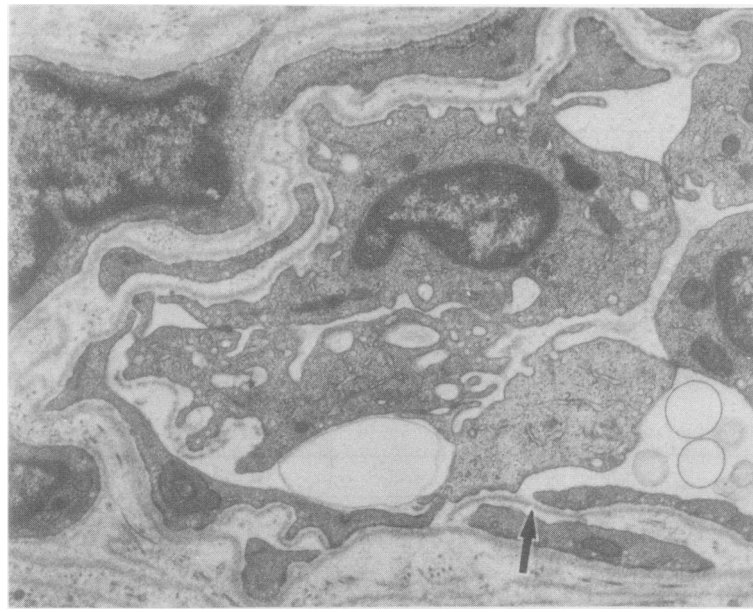

Fig. 2 *Electron micrograph of small blood vessel showing hyperplastic endothelial cells with separation of adjacent cell borders extending down to and affecting base of cells, which opens up possible channels of communication between lumen of vessel and vessel wall (arrow). ×12 500.*

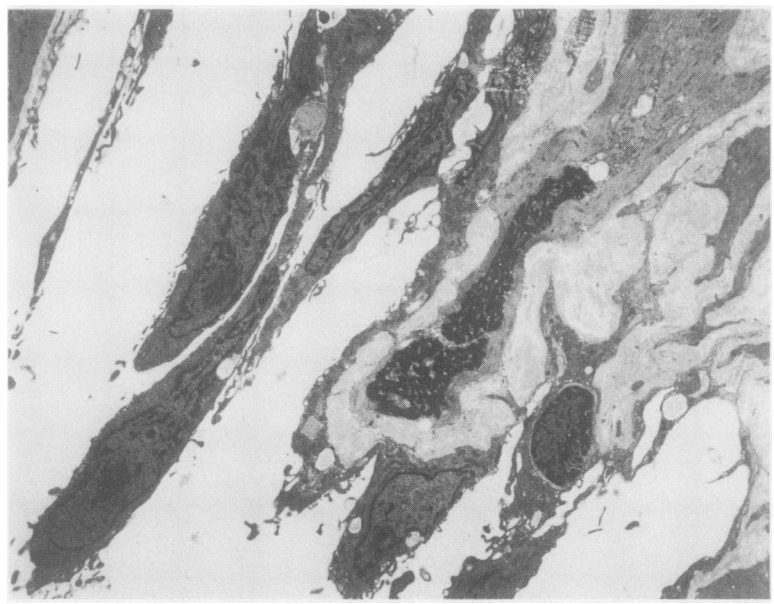

Fig. 3 *Electron micrograph of longitudinal section of blood vessel showing elongated endothelial cells extending into and along lumen of vessel. ×3000.*

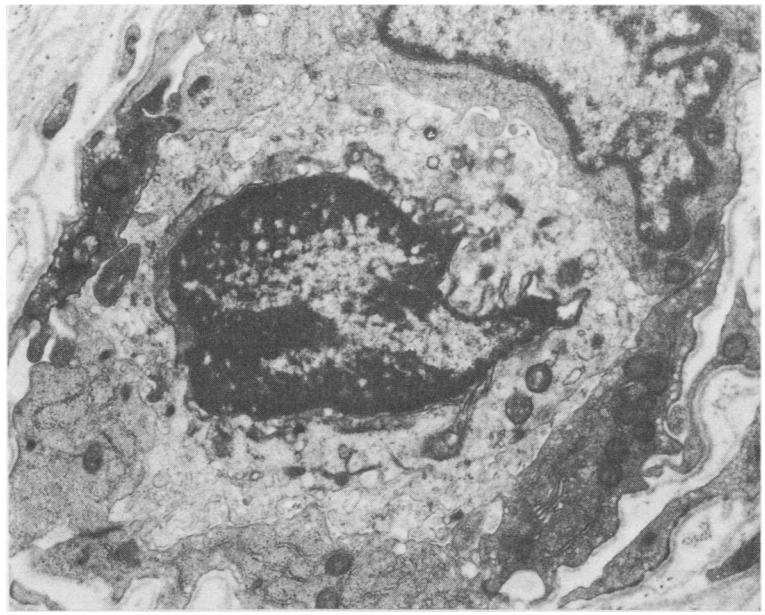

Fig. 4 *Electron micrograph of small blood vessel showing plugging of lumen with degenerate cell with considerably pyknotic nucleus. ×7500.*

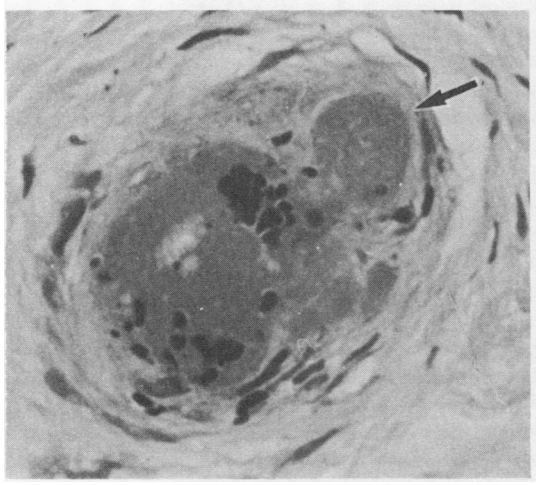

Fig. 5 *Section of diabetic sural nerve showing small blood vessel occluded by thrombus with fibrin tracking into vessel wall (arrow). Darkly staining cells within thrombus are red blood cells. (Solochrome cyanine stain.) ×1200.*

another vessel a group of degranulated platelets was attached to the vessel wall. In another case red blood cells were seen within the wall of the vessel (Fig. 7). Six of the diabetic cases showed pronounced pinocytosis (Fig. 8).

Electron microscopical examination of the control series showed hyperplasia of endothelial cells in only one patient; this was a man with carcinomatous neuromyopathy. All other patients with non-diabetic neuropathy had normal vessels. Vessels in the sural nerves of the six cases studied at necropsy were all normal with no evidence of endothelial cell hyperplasia, plugging of the vessel lumen with degenerate endothelial cells, thrombus, or platelets.

Discussion

The results presented above show abnormalities of small blood vessels in the sural nerves of 11 patients with diabetic neuropathy. These patients had severe progressive neuropathy, predominantly motor in type but sensory in one case. In all cases the glycosylated haemoglobin concentration indicated good metabolic control.

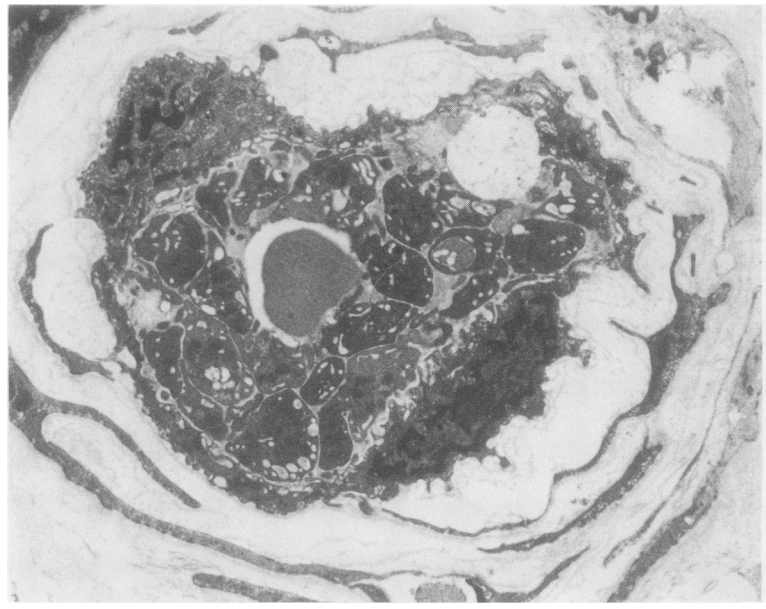

Fig. 6 *Electron micrograph of small blood vessel containing plug of degranulated platelets. ×7500.*

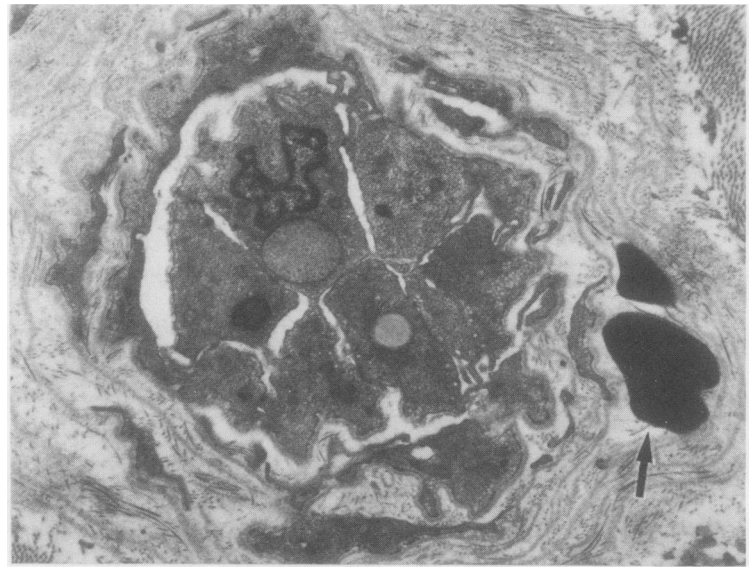

Fig. 7 *Electron micrograph of small blood vessel showing two red blood cells within wall of vessel (arrow). Note also hyperplastic endothelial cells almost occluding lumen of vessel. ×7500.*

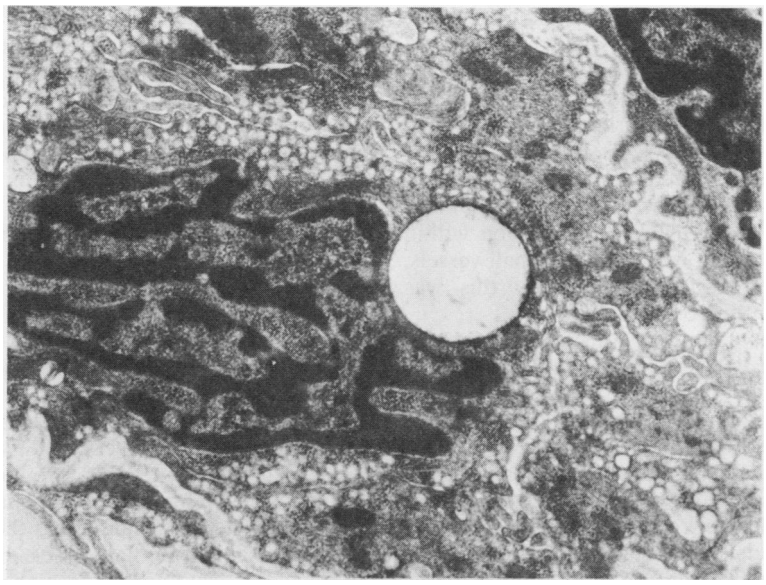

Fig. 8 *Electron micrograph of small blood vessel showing appreciable pinocytosis within hyperplastic endothelial cells almost occluding lumen of vessel.* ×*20 000.*

All cases showed considerable endothelial cell hyperplasia in some vessels sufficient almost to completely occlude the lumen of the vessel, and seven cases showed plugging of the vascular lumen by partially degenerate cellular material and electron dense protein, which was at least partly composed of unstriated fibrin. Five patients with non-diabetic neuropathy showed no evidence of endothelial cell hyperplasia or vascular plugging with degenerate cells, electron dense protein, or platelets.

Hyperplasia of endothelial cells does not seem to be specific for diabetes. In this series one patient with carcinomatous neuromyopathy also showed some vascular endothelial cell hyperplasia, and in a previous study by Williams *et al* hyperplasia of endothelial cells was noted in the sural nerve of a patient with an osteogenic sarcoma being treated with cytotoxic drugs.[5] It has also been described in hypertension.[6] It does, however, seem to be a strong feature of diabetic vessels, and it has been described in digest preparations of the retina of patients with diabetic retinopathy associated with degenerative changes in pericytes or complete absence of these cells.[7]

In a correlative study between digest preparations and fluorescence retinal angiograms Kohner and Henkind suggested that increased intravascular pressure may stimulate proliferation of endothelial cells as it does in collaterals after occlusion of the branch vein.[8] Once established it would result in stasis of flow. Ischaemia of the vessel wall and adjacent tissues, degenerative changes in the vessel wall, and desquamation of degenerate endothelial cells could result in plugging of the lumen at a more distal point of narrowing of the lumen. In addition, protrusion of hyperplastic endothelial cells into the lumen of a vessel and extension along the lumen between other hyperplastic endothelial cells could also result in occlusion. Interestingly, when vascular endothelial cell hyperplasia is most pronounced there is sometimes separation at the points of junction of adjacent endothelial cells, particularly at their base; this could result in breakdown of the endothelial cell barrier and result in seepage of plasma or even cellular components into the vessel wall and, in the long term, cause vascular thickening. In one case in this series red blood cells were seen within the wall of such a vessel.

In experimental studies on diabetic rats Slater *et al* showed that capillaries within diabetic nerves often show an increased amount of cytoplasm with more numerous pinocytotic vesicles and microvilli, and that hyperplasia of endothelial cells results in narrowing of the vessel lumen; these vessels become normal after treatment.[9] Such endothelial cell hyperplasia is seen within three to four weeks of the onset of diabetes in the rat. This could provide a

mechanism for disturbance of blood flow and neuronal function from the early stages of the diabetic state.

In the previous light microscopical study of sural nerve biopsies from diabetic patients Timperley *et al* showed plugging of small vessels with fibrin or thrombus, together with areas of necrosis and infarction, within nerve bundles.[4] Clumps of granular material thought to be platelets were seen within fibrin that had occluded the lumen of small vessels, and in one case studied at necropsy this was confirmed electron microscopically.

In an experimental study of proximal motor neuropathy in the BB Wistar rat Sima and Thibert showed endoneural infarcts of varying ages in the ventral nerve roots.[10] In the vicinity of partially organised infarcts, occluded arterioles could be shown. On electron microscopical examination the luminal material was found to be electron dense unstriated fibrin. Evidence of endothelial cell damage was observed and included vacuolation of endothelial cell cytoplasm and disruption of basement membranes. Leucocytes and fibrin were occasionally observed outside the vessels. More recent infarcts showed fusiform swellings of the fasicles with swollen myelinated fibres. In these roots several vessels showed occlusion by platelet aggregation similar to that seen in one of our cases. Electron microscopy showed that some of the platelets were in a secretory state and some were degranulated. Surrounding endoneural tissues showed considerable oedema and swelling of unmyelinated and myelinated axons, which were surrounded by attenuated myelin sheaths. In one affected root infarcts of varying ages could be distinguished. The endoneural vessels of unaffected roots showed thickening and duplication of basement membranes. In Sima and Thibert's study infarcts were not observed in sural, peroneal, tibial, or sciatic nerves, and they concluded that this form of neuropathy is structurally and most probably pathogenetically distinct from distal neuropathy. In the present study of small nerves one case showed occlusion of vessels with degranulated platelets and one case showed focal necrosis of nerve fibres, probably due to infarction, indicating that vascular lesions are also present in distal nerves.

Neurophysiological results in the present study showed that all diabetic patients had evidence of motor neuropathy, which in seven cases was moderate or severe. Changes were more pronounced distally than proximally and more severe in the leg than the arm. In two patients, however, there was disproportionately severe proximal denervation, and in one patient denervation was greater in the arm than in the leg. Severe sensory denervation was present in all but one patient. If the neuropathy in the group as a whole were related to multiple small infarcts then at least some patients should have shown a pattern of denervation that was not symmetrical and distally predominant. This was observed in three patients in whom the clinical and electrophysiological distribution of denervation was that of mononeuritis multiplex rather than generalised peripheral neuropathy. A further four patients showed this feature clinically though not electrophysiologically.

It is possible that after a long period of accumulation of multiple small ischaemic lesions an electrophysiological picture of diffuse polyneuropathy would be produced. It seems unreasonable to assume that an electrophysiological picture of diffuse polyneuropathy always indicates a metabolic defect as it could result from extensive focal lesions due to inadequate neuronal perfusion. Inadequate perfusion at capillary level could result in ischaemia of individual Schwann cells, producing a picture of segmental demyelination and axonal degeneration.

The present study emphasises the important role that abnormalities in small vessels may have in terms of damage to many tissues in diabetic patients, and we emphasise the importance of further work to clarify the interplay between metabolic and vascular factors.

We thank Mrs S Porter for her technical help and Mrs P Kirk for typing the manuscript.

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