ABNORMAL MICROVASCULATURE IN DIABETIC NEUROPATHY

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SUMMARY

Microvascular abnormalities are prominent in the retina and kidney in diabetic subjects with the specific complications of the disease. It would not therefore be surprising to find similar changes in nerve, the other tissue prominently damaged in diabetes. A variety of biochemical abnormalities have been postulated as a cause of nerve damage but so far none has been reversed with any benefit to patients. It is now very clear that major structural and functional changes in the microvasculature occur in diabetic neuropathy and are probably involved in resulting nerve damage. Endothelial thickening of the endothelial basement membrane occurs, as does luminal occlusion caused by endothelial cell proliferation; human sural nerve is hypoxic; sural nerve epineurial arterioles are attenuated and involved in arteriovenous shunting associated with gross distension and tortuosity of veins; flow of fluorescein is impaired in neuropathic nerves and nerve conduction increment on exercise is impaired in neuropathy. Therapeutic intervention should now be directed at these gross abnormalities.

Although the aetiology and pathogenesis of human diabetic neuropathy remains unclear, microvascular/hypoxic and metabolic mechanisms have been postulated. The case for microvascular disease in diabetic neuropathy is strong. Fibre loss in human sural nerve is multifocal, suggesting ischaemia. The degree of endoneurial vessel disease has been related to the severity of neuropathy. People with chronic obstructive airways disease develop so-called hypoxic neuropathy in which similar microvascular changes occur as in diabetic neuropathy. In rats with experimental diabetic neuropathy nerve blood flow is reduced and oxygen supplementation has improved deterioration in nerve conduction velocity. Similarly, in human diabetic neuropathy there is impaired nerve blood flow, epineurial arteriovenous shunting and a reduction in sural nerve oxygen tension. The stage during the development of nerve damage at which these changes occur is yet to be determined.

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Diabetic polyneuropathy leads to considerable morbidity and mortality.¹ Unpleasant sensory syndromes afflict approximately 10% of diabetic people and foot ulceration and infection result in many hospital admissions.² In the United States neuropathy takes its place in the estimated 35 000 amputations that occur annually.³ The incidence of diabetic neuropathy varies depending on diagnostic criteria.⁴ Despite the common clinical development of neuropathy the exact aetiology remains obscure. Undoubtedly poor glycaemic control plays a major part in nerve damage,^{5,6} but sorbitol accumulation in nerve coupled with myo-inositol depletion and a reduction in sodium-potassium-ATPase have all been postulated from work in animals. Non-enzymatic glycosylation of proteins, defective protein synthesis and abnormalities of axonal flow are also postulated as possible mechanisms. Of course it is possible that metabolic pathways have some effect on nerve vasculature.^{7,8}

MICROSCOPIC ABNORMALITIES OF NERVE BLOOD VESSELS IN DIABETIC NEUROPATHY

In recent years there has been a revival of the case for microvascular disease in diabetic neuropathy from both human and animal studies. The idea that diabetic neuropathy might be due to vascular disease has its roots in early studies by Pryce⁹ and later work by Woltman and Wilder.¹⁰ They attributed neuropathy to arteriosclerosis of vasa nervorum. However, some of the 'infarcts' described by Woltman and Wilder have the appearances of Renaut's bodies which are found in normal nerve.^{11,12}

Three decades later, Fagerberg drew attention to small vessel disease with thickening and hyalinisation of intra-neural vessel wall by a material staining PAS positive, together with a reduction in vessel calibre, in diabetic neuropathy.¹³ These changes were later found to be due to reduplication of capillary basal lamina,¹⁴ which in turn may be due to episodes of increased endothelial cell turnover; although found in other neuropathies,¹⁵⁻¹⁷ the phenomenon is commoner in diabetes.¹⁴

Several studies have since confirmed the presence of

increased basement membrane thickness in endoneurial capillaries in chronic diabetic neuropathy.^{7,17-19} In a study by Malik *et al.*⁷ endoneurial capillary basement membrane, endothelial cell and total diffusion barrier were significantly increased in diabetic neuropathy. These changes were found to be more severe in nerve capillaries compared with muscle or skin capillaries. Furthermore, the degree of endoneurial vessel disease has been related to the severity of both nerve fibre pathology and clinical neuropathy.⁷ This relationship has been reported by other studies.^{13,18,19}

Endothelial cell hyperplasia has been found in endoneurial capillaries in diabetic neuropathy,^{20,21} in some cases leading to complete occlusion of small vessels.²¹ However, endothelial cell hyperplasia has also been demonstrated in patients with type I hereditary motor and sensory neuropathy.¹⁶ In a study involving sural nerve biopsies from diabetic patients with progressive, mainly motor neuropathy, with good glycaemic control, Timperley *et al.*²² described plugging of small vessels by degenerate cellular material and electron-dense protein which was partly composed of fibrin. Endoneurial capillary closure has also been shown in patients with diabetic neuropathy,²³ but this has not been confirmed by other studies.^{16,24}

The diffuse symmetrical nature of many clinical syndromes of diabetic neuropathy has led to the postulate that the aetiology must be a diffuse metabolic abnormality. However, Dyck *et al.*²⁵ in a post-mortem study of transverse sections of nerve roots in the lower limb found that the spatial distribution of fibre loss in diabetic neuropathy suggested ischaemia. The same group in teasing nerve fibres of sural nerve demonstrated that fibre loss in diabetic neuropathy is primary and multifocal, again suggesting ischaemia.²⁶

EXPERIMENTAL ISCHAEMIC NEUROPATHY

The blood supply of the peripheral nerve is abundant. In a series of experiments performed on the dog sciatic nerve, Roberts²⁷ found that when the blood supply of a nerve was obliterated by ligating a single nutrient artery or nutrient arteries of a small segment, only partial or patchy ischaemia usually occurred because collateral anastomoses through the longitudinal vessels in the nerve could maintain adequate nutrition. This has since been confirmed by other studies.^{28,29} To occlude endoneurial vessels selectively, Nukada and Dyck^{29,30} injected microspheres into arteries supplying the sciatic nerve; they reported that many microvessels had to be occluded before fibre degeneration occurred. Benstead *et al.*³¹ ligated major pelvic arteries of rats in order to study the acute morphological effect of ischaemia on ultrastructural features of endoneurial microvessels. They observed that in ischaemic nerves there was an increase in endothelial cells, pericytes and basement membrane. The endothelial cells appeared swollen with abundant mitochondria. This study suggests that ischaemia has a direct effect on the

microvessel wall in addition to its well-established effect on nerve fibres.^{27,29}

Further animal studies strengthen the case for microvascular mechanisms in pathogenesis. Tuck *et al.* demonstrated reduced nerve blood flow and oxygen tension in rats with experimental diabetic neuropathy and Low *et al.* showed that supplementation with oxygen or the use of hyperbaric oxygen improved the deterioration in nerve conduction velocity.^{32,33} In a hypoxic environment normal rats developed electrophysiological³⁴ and morphological³⁵ abnormalities similar to those seen in experimental diabetes, while oxygen supplementation was able partially to prevent conduction slowing.³⁶

PATTERNS OF BLOOD FLOW AND OXYGEN TENSION IN HUMAN DIABETIC NEUROPATHY

The role of epineurial nutrient arterioles is obviously important, but has not been investigated in as much detail as that of endoneurial vessels. Korthals *et al.*³⁷ measured the epineurial arteriolar wall components in sural nerves of healthy controls and of diabetic subjects most of whom had neuropathy. Intimal area and numbers of intimal nuclei were significantly greater in diabetic subjects compared with controls. The authors concluded that the increased intima, and the possible resulting decrease in nerve perfusion, may contribute to the development of diabetic neuropathy, although they found no direct relationship between the increase in intima and severity of nerve fibre degeneration.

BLOOD FLOW AND OXYGEN TENSION IN THE DIABETIC PERIPHERAL NERVE

In human diabetic neuropathy although there is a reduction in sural nerve oxygen tension there is an elevation in oxygen tension in the foot veins due to arteriovenous shunting.³⁸ From other work it is well established that there is arteriovenous shunting in the diabetic neuropathic foot with large distended veins,^{28,29} and it seems that similar shunting is taking place in nerve itself. A novel *in vivo* technique has been developed for visualisation at high magnification of epineurial vessels in exposed sural nerves,²⁷ and following photography a technique of nerve fluorescein angiography has also been applied which allows the study of nerve blood flow in exposed sural nerve.³⁸ After intravenous administration of fluorescein its appearance in the nerve was monitored using a closed circuit television attached to an operating microscope. The fluorescein appearance time and intensity of fluorescence were quantified using computer analysis of video digitised images. The intensity of nerve fluorescence was markedly reduced in subjects with diabetic neuropathy compared with non-neuropathic diabetic and normal controls, suggesting impaired nerve blood flow in human diabetic neuropathy.

Haemorrhological abnormalities that have been described in diabetes may contribute to impaired blood flow brought about by microvascular disease.³⁹ Fibrin

deposition and platelet clamping have been observed in endoneurial vessels in diabetic neuropathy.^{20,40} The abnormalities of platelet function,⁴⁰ the increased blood and plasma viscosity,⁴¹⁻⁴³ the reduced red cell deformability,^{44,45} and the increased erythrocyte aggregability and adhesiveness,^{46,47} may lead to a reduction in capillary erythrocyte velocity.^{48,49} Episodes of sludging and stasis that may consequently develop, may, if severe, lead to local hypoxia.

The exact stage at which the structural and functional abnormalities of the microvasculature occur is yet to be established. However, gross microvascular abnormalities have been described in 5 patients developing acute painful neuropathy soon after the institution of blood glucose control by the use of insulin. *In vivo* studies as described above revealed leakage of fluorescein and gross abnormalities of the epineurial vessels with obvious arteriovenous shunting and leashes of small proliferating vessels identical to those described in proliferative diabetic retinopathy.⁵⁰

A POTENTIAL MARKER OF IMPAIRED NERVE BLOOD FLOW

Despite the availability of neurophysiological and quantitative sensory testing, we do not have a marker of early diabetic neuropathy or of impaired peripheral nerve blood flow (if this is indeed an important factor). A non-invasive test has been developed which may identify those diabetic subjects who will eventually develop neuropathy – clearly a prerequisite for early intervention.⁵¹ The test involves measuring sural sensory conduction velocity and subcutaneous temperature near the sural nerve before and after exercise on a treadmill to 80% maximal heart rate. Sural sensory conduction velocity increased significantly after exercise in normal subjects (mean increase 5.07 m/s) and non-neuropathic diabetic subjects (mean increase 3.99 m/s) but not in neuropathic subjects (mean increase 0.99 m/s). We have also found diabetic subjects with a Dyck's neuropathy score⁵² of 0 (i.e. no neuropathy) who are unable to increase their nerve conduction velocity after exercise. We speculate that these may be the diabetics who go on to develop clinical neuropathy. This will have to be confirmed by further studies, but if it proves to be the case, then this simple non-invasive test, which is within the means of most hospitals in the United Kingdom, may be regarded as an early marker of impaired nerve blood flow.

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