Letters to the Editors

Myelinated nerve fibres in experimental diabetes: a reply to Bhoyrul and Sharma

Sir,

A recent paper [1] elicited comments from Bhoyrul and Sharma [2] concerning methods we employed to characterise myelinated fibres. They believe that light microscopy cannot be used to measure variables relating to axon or myelin area. Before we refute their criticisms, we refer readers to a recent review [3] illustrating the sophisticated measurements possible by light microscopy, some of which were employed in our study. In addition, we have recently confirmed and extended the main finding in the original paper of myelin expansion using electron microscopy.

Bhoyrul and Sharma suggest that a magnification (1200 X) used was inadequate to allow measurements on fibres less than $20\text{ }\mu\text{m}^2$ in area. With the equipment and procedures employed in the study, the reproducibility of repeated area measurements was assessed. The maximum range of variability for the smallest ($2\text{ }\mu\text{m}$ diameter) fibres was 7%. This proved to be a constant error, so that its effect on measurements decreased as fibre size increased. As more than 2000 fibres were measured per experimental group, 0.16% is the maximum variability expected from this source, which is trivial compared to inter-animal or group variations [1].

One technical reason for not measuring detail on small fibres concerns section thickness. The thicker the section, the more myelin area is overestimated and axon area underestimated, reaching significance for small fibres with semithin sections. It is a geometric effect correctable by calibration [3] and would not affect between-group comparisons unless there were dramatic shifts in fibre calibre spectrum, which we did not observe.

Our primary reason for restricting attention to larger fibres was the specific hypothesis we were addressing. This involved a correlation of morphology with motor nerve conduction, and the specific interest was in α -motor fibres. By probability analysis we separated a large calibre population, containing both α -motor and large sensory fibres from small sensory and autonomic fibres. We did, incidental to the main question, examine the latter population; no diameter changes were found amongst the small fibres. We cannot accept that the exclusion of smaller fibres alters the validity of our results or conclusions.

Bhoyrul and Sharma question axonal area measurements on the basis that adaxonal Schwann cell cytoplasm would be included. The adaxonal space is small in peripheral nerves. From electron micrographs, we have calculated that its inclusion would generate a maximum 3% overestimate of axonal area on a representative sample. This would only affect between-group comparisons if Schwann cell cytoplasmic volume changed in experimental diabetes. Jakobsen [4] has shown that it decreased by 30%. Therefore, compared with normal controls, diabetic axon area would be underestimated by 1%, which is negligible compared with observed biological variability [1].

Bhoyrul and Sharma present data on axonal area which broadly support our conclusion that there is an axonal maturation deficit in diabetes. However, to argue that this is the only axonal effect ignores a

large body of evidence. Numerous studies using chemically-induced or spontaneously diabetic animals [5–8] demonstrate in the longer term (8–12 months) a progressive shrinkage, axonopathy, and eventual fibre loss [9].

Our estimates of myelin area and the myelin expansion in diabetes were criticised because we may not have discriminated myelin sheath from Schwann cell cytoplasm. This does not apply, as cytoplasm and axoplasm were only lightly stained in our sections and axon/myelin area ratios are in good agreement with the literature [10]. Again, there is no reason to expect between-group differences on this point.

We were also criticised for not taking account of “errors introduced by separation of myelin lamellae or unrecognised Schmidt-Lanterman incisures.” Schmidt-Lanterman incisures are easily recognisable. The point about lamellar separation or splitting is more important. Rather than reiterate arguments already made in our paper [1], we quote from a recent study by Mattingly and Fischer [5] using streptozotocin-diabetic rats, whose findings are in good agreement with our own “. . . myelin abnormalities which could produce an increase in the size of the myelin sheath including delamination, splitting, wrinkling and notching have been observed by many investigators . . .” [8, 11–14]. We did not think it was justifiable to exclude these badly affected areas or fibres from our sample, as to do so would have biased our estimate of myelin sheath area towards normality.

In mature rats, myelin expansion is evident from several studies using streptozotocin-diabetic [1, 5, 10, 15] or spontaneously diabetic [6, 16] models. Bhoyrul and Sharma introduce contradictory data; their diabetic rats had normal myelin areas for the axon calibres involved. It is difficult to comment in detail, as their methods and results have not been published in full. However, this contradiction is partly explicable if they had unjustifiably corrected for “errors” due to myelin delamination or splitting, and avoided measurements on fibres showing such characteristics in diabetic animals. To obtain an estimate of myelin sheath thickness, they appear to have multiplied the number of lamellae by a standard value for interlamellar spacing. This provides an inaccurate measure of sheath size, which may be further faulted if lamellar spacing undergoes changes with diabetes. However, this point alone cannot explain the discrepancy between their results and the body of the literature. In streptozotocin-diabetic rats [15] there was a 17% increase in lamellae number for a given axon size, and in BB-Wistar rats [6] after 8 months of diabetes there were 69% and 17% increases in lamellae for sural and peroneal nerves respectively.

We conclude that comments on the morphometric part of our study by Bhoyrul and Sharma do not survive critical examination, and that their own methodology may be seriously flawed.

Yours sincerely,
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References

1. Cameron NE, Leonard MB, Ross IS, Whiting PH (1986) The effects of Sorbinil on peripheral nerve conduction velocity, polyol concentrations and morphology in the streptozotocin-diabetic rat. *Diabetologia* 29: 168–174
2. Sharma AK, Bhoyrul S (1986) Myelinated fibre population in experimental diabetes. *Diabetologia* 29: 405 (Letter)
3. Dyck PJ, Karnes J, Lais A, Lofgren EP, Stevens JC (1984) Pathological alterations of the peripheral nervous system in humans. In: Dyck PJ, Thomas PK, Lambert EH, Bunge R (eds) *Peripheral Neuropathy*, 2nd edn. Saunders, Philadelphia, pp 760–870
4. Jakobsen J (1978) Peripheral nerves in early experimental diabetes. Expansion of the endoneurial space as a cause of increased water content. *Diabetologia* 14: 113–119
5. Mattingly GE, Fischer VW (1985) Peripheral nerve axon dwindling with concomitant myelin sheath hypertrophy in experimentally induced diabetes. *Acta Neuropathol (Berl)* 68: 149–154
6. Sima AAF, Bouchier M, Christensen H (1983) Axonal atrophy in sensory nerves of the diabetic BB-Wistar rat: a possible early correlate of human diabetic neuropathy. *Ann Neurol* 13: 264–272
7. Robertson DM, Sima AAF (1980) Diabetic neuropathy in the mutant mouse (C57/Ks(db/db)). A morphometric study. *Diabetes* 29: 60–67
8. Yagihashi S, Nishigira M, Baba M (1979) Morphometric analysis of the peripheral nerve lesions in experimental diabetes rats. *Tohoku J Exp Med* 129: 139–149
9. Powell HC, Myers RR (1984) Axonopathy and microangiopathy in chronic alloxan diabetes. *Acta Neuropathol (Berl)* 65: 128–137
10. Jakobsen J (1979) Early and preventable changes of peripheral nerve structure and function in insulin-deficient diabetic rats. *J Neurol Neurosurg Psychiatry* 42: 509–518
11. Mattingly GE, Fischer VW (1983) Peripheral neuropathy following prolonged exposure to streptozotocin-induced diabetes in rats: a teased nerve fiber study. *Acta Neuropathol (Berl)* 59: 133–138
12. Moore SA, Peterson RG, Felten DL, O'Connor BL (1982) Ultrastructural axonal pathology in experimentally diabetic and ageing control rats. *Brain Res Bull* 8: 317–323
13. Sima AAF, Lorusso AC, Thibert P (1982) Distal symmetric polyneuropathy in the spontaneously diabetic BB-Wistar rat. An ultrastructural and teased fibre study. *Acta Neuropathol (Berl)* 58: 39–47
14. Sima AAF, Robertson DM (1979) Peripheral neuropathy in the diabetic mutant mouse. An ultrastructural study. *Lab Invest* 40: 627–632
15. Sugimura K, Windebank AJ, Natarajan V, Lambert EH, Schmid HHO, Dyck PJ (1980) Interstitial hyperosmolarity may cause axis cylinder shrinkage in streptozotocin diabetic nerve. *J Neuropathol Exp Neurol* 39: 710–721
16. Yagihashi S, Tonosaki A, Yamada K, Kakizaki M, Goto Y (1982) Peripheral neuropathy in selectively inbred spontaneously diabetic rats: electrophysiological, morphometric and freeze-replica studies. *Tohoku J Exp Med* 138: 39–48

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