Editorial

Polyols and *myo*-Inositol in Diabetic Neuropathy—of Mice and Men

The discovery that peripheral nerves of diabetic rats accumulate sorbitol and fructose, as a result of increased flux of glucose through the enzyme aldose reductase, was made more than 20 years ago.[1] Concomitant depletion of free *myo*-inositol in nerve was reported at the same time,[1] but a link between the two phenomena was not forged until the demonstration that administration of inhibitors of aldose reductase to diabetic rats prevented not only accumulation of sorbitol and fructose in peripheral nerve but also depletion of *myo*-inositol.[2,3] Even so, the mechanism (or mechanisms) responsible for this link remains to be determined. The suggestion that depletion of *myo*-inositol in nerves may contribute to the development of conduction deficits in diabetic rats originated with the observation that these deficits did not develop if the depletion was precluded by dietary supplementation of *myo*-inositol.[2,4] Thus, a hypothesis has been formulated that suggests that depletion of *myo*-inositol might restrict turnover of inositol-containing lipids. It is further suggested that this, in turn, might restrict the availability of one of the second messengers derived from hydrolysis of inositol-containing lipids—namely, diacylglycerol. The next step is the suggestion that diacylglycerol might be responsible for promoting phosphorylation of the nerve membrane Na^+-K^+ ATPase (adenosine triphosphatase) by means of activation of protein kinase C. Therefore, it is implicit that restricted production of diacylglycerol in diabetic nerve would lead to impaired activation of the membrane Na^+-K^+ ATPase pump. The proposed consequences of this are deformation of the node of Ranvier and conduction disturbances. (Greene and associates[5] reviewed this hypothesis, and more information is provided by Dyck and colleagues in this issue of the *Proceedings*, pages 905 to 910.)

Attempts have been made to test the critical biochemical steps in this hypothesis, and rat and rabbit nerve models have contributed some experimental support. The problem of determining whether the hypothesis can explain phenomena in the nerves of patients, however, has received little attention, and studies to date are not supportive. Those investigations that have examined *myo*-inositol levels in fresh human nerve tissue have found no definite depletion, as represented by group mean values.[6-8] Although nerves from patients with diabetes show variability, correlation analysis showed no relationship between nerve *myo*-inositol levels and indices of the severity of neuropathy.[8] The study by Dyck and co-workers in this issue of the *Proceedings* was performed to test the possibility that fasting of patients before biopsy might mask a depletion of *myo*-inositol present in the nonfasting state. This possibility was addressed, in a study in rats, by obtaining nerves at different times after withdrawal of food. The data clearly show that levels of *myo*-inositol in the nerves of diabetic rats were lower 20 hours after feeding than at shorter times (1.5 or 4 hours) after withdrawal of food. Thus, the results cannot explain the discrepancy, and proposals of dysfunction derived from reduced levels of free *myo*-inositol in peripheral nerve apparently cannot be applied to human nerve, even though such hypotheses may be cogent to explain defects in rats.

The value of hypotheses is measured by the extent to which they stimulate further work. The proposals implicating *myo*-inositol in the development of dysfunction in diabetic nerve have already prompted many useful experiments and will provoke more. It is timely, however, to review the potential directions of such studies. Clearly, more information about human nerve is needed. Ethical and practical difficulties limit this investigative avenue, but those centers that

Address reprint requests to Professor D. R. Tomlinson, Department of Pharmacology, Medical College of St. Bartholomew's Hospital, Charterhouse Square, London EC1M 6BQ, UK.

can use this material have clear directions for fruitful studies. Some investigators have argued that changes in nerve Na^+-K^+ ATPase activity are central to axonal dysfunction, at least in short-term diabetes.[5] Measurement of this factor in vitro in sural nerve biopsy specimens is entirely feasible if the material is available and if operating room-to-laboratory transfer can be organized efficiently. Most studies to date have measured ouabain-sensitive ATPase activity by classic enzymatic assay with use of broken cell preparations. This technique yields extensive variability, even when used by experienced investigators.[9] Furthermore, application of this method to homogenates of rat sciatic nerve generates a nonlinear ATP hydrolysis that presumably reflects ouabain-sensitive and ouabain-insensitive ATPases in membrane fragments from all cell types contributing to the homogenate (the methodologic details are described elsewhere[10,11]). Because putative regulation of enzyme activity by protein kinase C is central to the outlined hypothesis, the methods of study used should preserve cellular relationships as rigorously as possible. A better approach has been adopted by others, who have examined ouabain-sensitive ^{86}Rb uptake into intact cells of the endoneurium.[12] Of course, this procedure makes no distinction between activity in axons and in Schwann cells, but at least the method registers ion pumping of an accredited potassium surrogate in intact cells. Of note, the application of this procedure to endoneuria from nerves of diabetic rats has revealed a deficit in ouabain-sensitive ^{86}Rb pumping. The deficit, however, differs in nature from that registered by enzymatic procedures applied to nerve homogenates.[12] Furthermore, this study revealed no difference between diabetic and control nerves in the steady-state activity of cytosolic protein kinase C. An examination of the activated membrane component of the enzyme would, however, be of greater relevance to the hypothesis. Nevertheless, the point to be made is that valid methods exist to examine the currency of this hypothesis, and their application to fresh human peripheral nerve would be of considerable value.

The laboratory rat—either streptozotocin-induced diabetic or spontaneously diabetic (BB Wistar)—has become the species of choice for most groups studying animal models of diabetic neuropathies. The aforementioned considerations, however, dictate that further work must explore other models in an attempt to discover diabetes-related changes in nerve biochemistry that resemble more closely those seen in human nerve. Of course, not enough is known about the changes in human nerve to allow precise modeling, even if that were possible.

As previously mentioned, we need more information about biochemical interactions in human nerve, but work with animal models continues to produce interesting and relevant surprises. For example, experiments performed in my laboratory indicate that changes produced by exaggerated flux through aldose reductase in the nerves of laboratory mice may be of relevance to the state of affairs in human nerve. Superficial examination of this area of biochemistry in mice indicated that the species possessed no enzyme with aldose reductase-like activity. In the diabetic animals in our colony of C57BL/Ks (db/db) mice, lenticular cataracts never developed. Experiments with nerves from spontaneously diabetic mice showed no pronounced accumulations of either sorbitol or fructose and no depletion of *myo*-inositol at durations of diabetes of 8 to 14 weeks.[13] In addition, no conduction deficits were noted in motor nerves after modest durations of diabetes. In nondiabetic littermates, diabetes was induced with streptozotocin, and similar measurements were made 3 weeks later. Again, absence of accumulations of sorbitol or fructose, no depletion of *myo*-inositol, and no deficit in motor nerve conduction velocity were noted.[13] Thus, we suggested the absence of aldose reductase activity from the nerve.

In a later study, we examined the biochemical changes in mouse nerve associated with feeding of galactose. Administration of a 20% galactose diet to nondiabetic or diabetic (C57BL/Ks) mice for only 5 days caused substantial accumulation of dulcitol in the sciatic nerve, but no associated depletion of *myo*-inositol was evident. Indeed,

the galactose-fed mice showed a small, but statistically significant, increase in nerve *myo*-inositol. Administration of the aldose reductase inhibitor ponalrestat with the galactose diet prevented accumulation of dulcitol.[14] Dulcitol, formed by the action of aldose reductase on galactose, is not metabolized by sorbitol dehydrogenase; therefore, it accumulates more rapidly and attains higher levels than does sorbitol. Accumulation of dulcitol is associated with notable depletion of *myo*-inositol in the nerves of galactose-fed rats;[1] thus, the link between accumulation of polyols and depletion of *myo*-inositol extends beyond sorbitol in this species. We suggested that this link is absent in the nerves of mice.[14] We argued further that the lack of accumulation of sorbitol in the nerves of diabetic mice (fed a normal diet)[13] does not preclude considerable flux of glucose through aldose reductase; it could simply arise from increased affinity or activity (or both) of sorbitol dehydrogenase, thereby clearing sorbitol as fast as it is formed, plus an increase in clearance of fructose. This sequence of events, however, would not explain the lack of depletion of *myo*-inositol inasmuch as levels of the latter were normal in nerves of diabetic and galactosemic mice, despite accumulation of dulcitol. Clearly, therefore, the mouse is a species in which accumulation of polyols in nerve is uncoupled from depletion of *myo*-inositol, in which respect it perhaps resembles humans. Obviously, the pattern of change in mouse nerve is extreme because of the virtual absence of sorbitol in nerves from diabetic animals. It is worth noting, however, that levels of sorbitol in human nerve seem to be much lower, relative to nerve mass, than those in rat nerve.[6-8]

Studies performed in my laboratory early this year in collaboration with Biswas and Calcutt make these phenomena even more interesting. Because dulcitol accumulates in nerve of galactose-fed mice and no concomitant depletion of *myo*-inositol occurs, this model can be used to answer questions that relate these biochemical changes to nerve conduction and deficits in Na^+-K^+ ATPase activity. Accordingly, we performed experiments to compare female mice (SAS/4 strain; 29 to 34 g body weight) that were fed a diet containing 20% galactose with age- and weight-matched control animals given a normal diet. The diet was administered for 4 weeks, after which motor nerve conduction velocity was measured by using standard methods[2] with the nerve maintained at 37°C. Mice were then killed 3 days later, and ATPase activity was measured in homogenates made from one sciatic nerve exactly as described elsewhere,[11] except that the ouabain concentration used for Na^+-K^+ ATPase activity was 0.2 mM. The other sciatic nerve was extracted and assayed for monosaccharides and polyols, again with use of established methods.[11]

On the basis of the data shown in Table 1, it can be seen that feeding of galactose to mice caused accumulation of dulcitol in the sciatic nerve but did not deplete free *myo*-inositol. These findings were associated with an appreciable slowing of motor nerve conduction velocity, but no deficit was noted in either composite or ouabain-sensitive ATPase activity. The following conclusions can be drawn. A biochemically derived conduction deficit can be generated with the use of galactose, without depletion of nerve *myo*-inositol or interference with Na^+-K^+ ATPase activity. This deficit is probably related to the accumulation of dulcitol, but that conclusion is not warranted until the conduction deficit has been prevented with an aldose reductase inhibitor. Thus, although these data have no influence on putative relationships between *myo*-inositol and ATPase defects, they do not support the theory that acute conduction deficits in diabetes are related to either phenomenon. If no depletion of *myo*-inositol is found in human nerve, then a similar situation might pertain. As has been emphasized in this editorial, links between nerve *myo*-inositol levels and dysfunction in diabetic nerve remain something of a mystery, and more work involving new directions must be done.

David R. Tomlinson, Ph.D.
Department of Pharmacology
Medical College of St. Bartholomew's Hospital
London, United Kingdom

Table 1.—**Nerve Content of Galactose and Dulcitol, Motor Nerve Conduction Velocity, and Total and Ouabain-Sensitive ATPase Activity in Sciatic Nerves of Control and Galactose-Fed Mice***

Factor	Control mice	Galactose-fed mice†	P‡
Sciatic nerve content (nmol/mg wet nerve)			
Galactose	0.09 ± 0.06	2.27 ± 0.63	<0.01
Dulcitol	0.07 ± 0.04	5.07 ± 0.34	<0.001
Motor nerve conduction velocity (m/s)	64.2 ± 3.8	48.1 ± 1.9	<0.01
ATPase activity (nmol/ATP/h/μg protein)			
Total	9.51 ± 0.56	8.65 ± 0.63	NS
Ouabain-sensitive (0.2 mM)	2.65 ± 0.44	2.81 ± 0.24	NS

*ATPase = adenosine triphosphatase; NS = not significant. Data are shown as means ± SEM; each group consisted of 9 mice.
†A diet of 20% galactose was fed for 4 weeks.
‡Group means were compared with use of unpaired *t* tests.

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