Impaired Rat Sciatic Nerve Sodium-Potassium Adenosine Triphosphatase in Acute Streptozocin Diabetes and its Correction by Dietary *Myo*-Inositol Supplementation

DOUGLAS A. GREENE and SARAH A. LATTIMER, *Diabetes Research Laboratories of the Department of Medicine, School of Medicine, University of Pittsburgh, Presbyterian-University Hospital, Pittsburgh, Pennsylvania 15261*

A B S T R A C T   Nerve conduction impairment in ex-perimental diabetes has been empirically but not mechanistically linked to altered nerve *myo*-inositol metabolism. The phospholipid-dependent membrane-bound sodium-potassium ATPase provides a potential mechanism to relate defects in diabetic peripheral nerve *myo*-inositol-phospholipid metabolism, impulse conduction, and energy utilization. Therefore, the ef-fect of streptozocin-induced diabetes mellitus and di-etary *myo*-inositol supplementation on rat sciatic nerve sodium-potassium ATPase was studied. ATPase activity was measured enzymatically in sciatic nerve homogenates from 4-wk streptozocin diabetic rats and age-matched controls either fed a standard or 1% *myo*-inositol supplemented diet. The sodium-potassium ATPase components were assessed by ouabain inhi-bition or the omission of sodium and potassium ions. Diabetes reduced the composite ATPase activity re-covered in crude homogenates of sciatic nerve. The 40% reduction in the sodium-potassium ATPase was selectively prevented by 1% *myo*-inositol supplemen-tation (which preserved normal nerve conduction). Thus, in diabetic peripheral nerve, abnormal *myo*-ino-sitol metabolism is associated with abnormal sodium-potassium ATPase activity. The mechanism of the ef-fect of dietary *myo*-inositol to correct diabetic nerve conduction may be through changes in a sodium-po-tassium ATPase, possibly via changes in *myo*-inositol-containing phospholipids.

INTRODUCTION

Nerve conduction is impaired in overt diabetic neu-ropathy by a combination of structural and metabolic defects in peripheral nerve (1, 2). Furthermore, the histologically demonstrable axonal degeneration and segmental demyelination are thought to reflect long-standing metabolic derangements in diabetic periph-eral nerve Schwann cells and/or axons (2). These un-known metabolic abnormalities alone presumably explain the acute and rapidly reversible conduction impairment in newly diagnosed human diabetes (2).

Nerve conduction defects in acutely diabetic ani-mals resemble the metabolically mediated conduction impairment in human diabetics (3–6). In the acute streptozocin diabetic rat, where diabetic nerve metab-olism and function are readily compared, impaired nerve conduction has been linked to an alteration in peripheral nerve *myo*-inositol (MI)[1] metabolism (3, 7, 8) that results from insulin deficiency and/or hyper-glycemia (3) (an analogous alteration in MI metabo-lism was recently demonstrated in human diabetic nerve [9]). In vitro studies with rabbit peripheral nerve suggest that competitive inhibition of sodium-depen-dent MI uptake by hyperglycemic glucose concentra-tions may contribute to this fall in diabetic nerve MI content (10), as may increased polyol-pathway metab-olism (8, 11, 12).

The relationship between altered MI metabolism and impaired nerve function is poorly understood. Recent single-node voltage-clamp studies in peripheral nerve from the spontaneously-diabetic BB rat ascribe impaired nerve conduction to a reduction in the rest-ing axonal transmembrane sodium potential, possibly attributable to reduced sodium-pump activity (13). The sodium-potassium ATPase, which generates the transmembrane sodium gradient in peripheral nerve, consumes the major fraction of the tissue's energy flux (14). Impaired resting energy utilization is a prominent metabolic defect in diabetic peripheral nerve (15).

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[1] *Abbreviations used in this paper:* MI, *myo*-inositol.

Furthermore, Das et al. (16) documented a reduction in sciatic nerve sodium-potassium ATPase activity in chronic streptozocin diabetic rats, but did not explore its possible metabolic relationships to insulin deficiency or hyperglycemia. Since conduction impairment in the acutely streptozocin diabetic rat is corrected by normalization of peripheral nerve MI metabolism (3, 7, 8), and since endogenous MI-containing phospholipids may modulate sodium-potassium ATPase in some tissues (17), we hypothesized that abnormal MI metabolism might underlie a sodium-potassium ATPase defect in diabetic peripheral nerve (18). This line of speculation was previously suggested by Clements (2), and may be strengthened by the recent observation that peripheral nerve free MI levels influence the metabolism of MI-containing phospholipids (19).

Therefore, we measured sodium-potassium-activated and ouabain-inhibited ATPase enzymatically in sciatic nerve homogenates from normal and streptozocin diabetic rats fed either normal or MI-supplemented diets (3, 7). Acute streptozocin-induced diabetes produced a 40% reduction in sodium-potassium-activated and ouabain-inhibited ATPase activity. This defect was selectively and completely prevented by the dietary 1% MI administration that normalizes nerve MI content and conduction velocity in streptozocin diabetic rats (3, 7).

METHODS

Animal model. Caesarian-delivered barrier-sustained male Wistar rats, initial weight 180–200 g, were fed and given water ad lib. throughout the 4-wk study. Animals received either a standard 0.011% MI (wt/wt) synthetic diet or a 1% MI synthetic diet, both consisted of pellets composed of 18% vitamin-free casein, 68% sucrose, 10% vegetable oil, 4% inorganic salts, and all known rat vitamin requirements (Nutritional Biochemical Corp., Cleveland, OH) (3, 7). To induce diabetes, streptozocin (Upjohn Co., Kalamazoo, MI), 60 mg/kg, was injected in 0.10 ml of 0.01 M citrate buffer, pH 5.5, into the tail vein of rats fasted overnight (nondiabetic controls received equal volumes of buffer alone). Streptozocin-treated rats with nonfasting plasma glucose concentrations < 300 mg/dl at 24 or 48 h after injection (heparinized tail-vein blood) or at death (heparinized cardiac blood) were excluded from the study.

Tissue and plasma collection. After 4 wk, nonfasted animals were anesthetized with 30–40 mg/kg i.p. sodium pentobarbitol. Midthigh segments of left and right sciatic nerves were surgically exposed with preservation of hemostasis, dissected free from contaminating extraneural connective tissue as previously described (3), rapidly weighed on a microbalance (final weight ∼ 20 mg/nerve), and processed either for enzymatic ATPase studies, or snap-frozen in liquid nitrogen partially evacuated to its freezing point (20) for subsequent gas-liquid chromatographic determination of MI content as described below. Cardiac blood was then obtained percutaneously, and the animal killed by a lethal dose of sodium pentobarbitol.

Analytical techniques. Plasma glucose was determined in a Beckman Glucose Analyzer II (Beckman Instruments, Inc., Fullerton, CA) (3).

Plasma and sciatic nerve MI were determined by gas-liquid chromatography in protein-free Somogyi filtrates of cardiac blood plasma or sciatic nerve homogenates as previously described (3) and expressed per milliliter of plasma or per gram wet weight of whole nerve (3, 7). The determinations of MI were performed on trimethylsilyl derivatives of lyophilized aliquots of the plasma or nerve filtrates containing alpha-D-methyl mannopyranoside as an internal standard in a Varian 3700 gas-liquid chromatograph on a 6 ft × 4 mm 3% SE-30 Gaschrom Q glass column at 185°C with a nitrogen carrier-gas flow rate of 40 ml/min using a flame ionization detector maintained at 250°C. Standard curves were determined daily, and the recovery of added MI to nerve or plasma samples consistently exceeded 95%.

Sodium-potassium activated and ouabain-inhibited ATPase activity was measured in freshly prepared crude homogenates of whole sciatic nerve and expressed per gram wet weight or milligram Lowry protein (21). Fresh sciatic nerve segments were minced and homogenized at 40°C in 2 ml of 0.2 M sucrose-0.02 M Tris–HCl buffer solution pH 7.5, using a Polytron homogenizer (model PT 10-35, Brinkman Instruments, Inc, Westbury, NY), for at least three periods, not exceeding 15 s each (16) and centrifuged at 100 g for 2 min at 4°C. 5–20 μl of supernatant was added to 1.0 ml × 1.0 cm disposable cuvettes (Fisher Scientific, Pittsburgh, PA) containing (final concentration) 100 mM NaCl, 10 mM KCl, 2.5 mM MgCl$_2$, 1 mM Tris–ATP, 1 mM tri(cyclohexylammonium) phosphoenolpyruvate, 30 mM imidazole-HCl buffer (pH 7.3), 0.15 mM NADH, and 50 μg of lactate dehydrogenase and 30 μg of pyruvate kinase (22). After initial stabilization, the oxidation of NADH was monitored at 340 nm in a Cary 210 Spectrophotometer in the dual-beam mode, and was linear with respect to time for at least 45 min. Sodium-potassium-activated ATPase activity was computed by subtracting (a) the reaction rate in an identical cuvette from which NaCl and KCl were omitted from (b) the reaction rate in the cuvette containing both of these salts (preliminary studies indicated that identical results were obtained if sodium alone were omitted). Ouabain-inhibited ATPase activity was measured by comparing the reaction rate before and after the addition of 0.10 mM ouabain (final concentration). (Preliminary experiments indicated that the final reaction rate was identical in cuvettes in which ouabain was added initially or after an initial linear rate was obtained in the absence of ouabain. Furthermore, 0.1 mM ouabain produced maximal inhibition within the concentration range of 0.05–1.0 mM ouabain.) The addition of ouabain had no effect on the apparent rate of the reaction in the absence of both sodium and potassium ions. Both ouabain-inhibited and sodium-potassium–stimulated activities were linear with time for at least 45 min and proportional to protein concentration between 2.5 and 15 μg/ml. Addition of MI (3 nmol) to the cuvettes, calculated to exceed the concentration of MI in sciatic nerve homogenates from MI-supplemented animals, did not alter the apparent rate of composite, ouabain-inhibited or sodium-potassium stimulated ATPase activity in diabetic or nondiabetic nerve homogenates. No additional ouabain-inhibited or sodium-potassium stimulated ATPase activity was recovered when the pellet resulting from the 100-g centrifugation (containing ∼6% of total Lowry protein) was resuspended in sucrose buffer and resonicated.

All reagents were obtained from Sigma Chemical Co. (St. Louis, MO) and were of the highest available purity unless otherwise stated.

RESULTS

Effect of streptozocin diabetes and dietary MI on body weight, plasma glucose, and plasma and sciatic nerve MI (Table I)

Dietary MI supplementation and 4-wk streptozocin diabetes produced the expected effects on final body weight, plasma glucose, and plasma and nerve MI concentrations (3, 7). The 1% MI diet had no detectable effect on nondiabetic or diabetic rat body weight, while experimental diabetes significantly lowered final body weight by ~40% in both dietary groups. Conversely, dietary MI had no detectable effect on final nondiabetic or diabetic plasma glucose concentration, while experimental diabetes raised plasma glucose in both dietary groups. Experimental diabetes produced no significant alteration in plasma MI in either dietary group, while the 1% MI diet raised final nonfasting plasma MI almost sevenfold in both nondiabetic and diabetic animals.

Effect of streptozocin diabetes and dietary MI on sciatic nerve ATPase activity (Table II)

Composite ATPase activity (column 1). Sciatic nerve homogenate composite ATPase activity, expressed per gram wet weight of nerve, was signifi-

cantly reduced in diabetic rats in both dietary MI groups. The 1% MI diet did not affect nondiabetic composite sciatic nerve ATPase activity. Although composite ATPase activity tended to be slightly higher in MI-supplemented diabetic rats than in diabetic rats fed the standard diet, this effect did not achieve statistical significance ($0.05 < P < 0.1$).

Ouabain-inhibited ATPase activity (column 2). Ouabain (0.1 mM) inhibited nondiabetic composite ATPase activity in both dietary groups by 21–22%; the absolute magnitude of the ouabain-inhibited ATPase fraction was thus not altered by dietary MI in nondiabetic rat sciatic nerve. Experimental diabetes diminished the ouabain-inhibited ATPase component by 40% in animals fed the standard diet (compare lines 1 and 3). The 1% MI diet increased ouabain-inhibited ATPase activity by 54%, so that it was no longer significantly smaller than in nondiabetic rats in either dietary group.

Sodium-potassium–stimulated ATPase activity (column 3, Fig. 1). The absence of sodium and potassium ions inhibited composite ATPase activity from sciatic nerve homogenates to the same degree as did the presence of 0.1 mM ouabain in each experimental condition. Thus, ouabain-inhibited ATPase activity and sodium-potassium–stimulated ATPase activity were of similar magnitude in each group, and were similarly affected by experimental diabetes and dietary MI (compare columns 2 and 3). Dietary MI did not affect nondiabetic sodium-potassium–stimulated ATPase activity; experimental diabetes reduced sodium-potassium–stimulated ATPase activity by 43%

TABLE I

Effect of Streptozocin Diabetes and Dietary MI on Rat Body Weight, Plasma Glucose and MI Concentration, and Sciatic Nerve MI Content

Animal/diet	n	Body weight	Plasma glucose	Plasma MI	Sciatic nerve MI
		g	*mg/dl*	*µM*	*mmol/kg*
Nondiabetic/					
Standard	(18)	364±8	178±7	34.5±1.7	2.98±0.20⌐
1% MI	(9)	354±7	164±5	228±31	⌐3.88±0.39
					P < 0.005
Diabetic/					
Standard	(20)	213±10	566±30	35.1±2.8	2.21±0.08⌐
					NS
1% MI	(18)	223±11	600±32	245±27	⌐3.93±0.23

After the 4-wk study period, animals were anesthetized with sodium pentobarbital 30–40 mg/kg i.p. and weighed. Blood for plasma glucose and MI was obtained by cardiac puncture and processed as described in Methods. One sciatic nerve was removed for ATPase determination as described in Methods and Table II. The contralateral nerve was removed, dissected free of contaminating non-neural connective tissue, weighed on a microbalance, frozen in liquid N_2, and homogenized and deproteinized with $ZnSO_4$/$Ba(OH)_2$ as previously described (3, 7) for gas-liquid chromatographic determination of MI as described in Methods. Values are expressed as mean±SEM.

TABLE II
Effect of Streptozocin Diabetes and Dietary MI on Rat Sciatic Nerve ATPase Activity

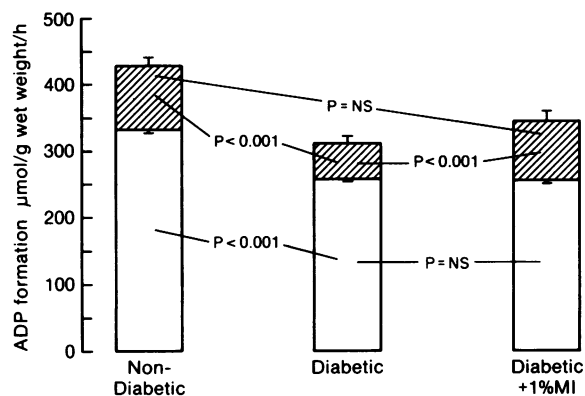|		ATPase activity			
Animal/Diet	n	Composite	Ouabain inhibited	Sodium-potassium stimulated	Nonouabain inhibited
		μmol/g wet wt/h			
Nondiabetic/					
Standard	(18)	429±12	96.8±3.8	97.3±4.5	333±11
		NS	NS	NS	NS
1% MI	(9)	423±27	91.5±5.8	91.5±5.8	331±25
		$P < 0.001$ $P < 0.001$	NS $P < 0.001$	NS $P < 0.001$	$P < 0.001$ $P < 0.001$
Diabetic/					
Standard	(20)	312±11	57.5±2.9	55.3±3.3	254±9
		NS	$P < 0.001$	$P < 0.001$	NS
1% MI	(18)	346±15	88.4±3.5	87.5±3.8	258±13

ATPase activity was measured enzymatically (20) in sciatic nerve crude homogenates from normal and 4-wk streptozocin diabetic rats fed either a standard or 1% MI supplemented diet. Ouabain-inhibited ATPase activity denotes the differential rate of ADP generated upon addition of 0.10 mM ouabain to the reaction mixture; non–ouabain-inhibited ATPase activity denotes residual ADP generation in the presence of ouabain. Sodium-potassium–stimulated ATPase activity indicates the differential rate of ADP generation when sodium and potassium ions are omitted from the reaction mixture. Values are mean±SEM, and P values are computed with the student's two-tailed t test (21).

in the absence of dietary MI supplementation; and the 1% MI diet raised diabetic sodium-potassium–stimulated ATPase activity by 58%, so that it no longer differed significantly from the nondiabetic in either dietary group. Thus, both ouabain inhibition and sodium-potassium stimulation appeared to define the same ATPase activity in sciatic nerve homogenates, i.e., the sodium-potassium ATPase.

Nonouabain-inhibited ATPase activity (column 4). Nonouabain-inhibited ATPase activity (i.e., residual activity in the presence of inhibitory concentrations of ouabain) was significantly lowered by experimental diabetes, but was unaffected by dietary MI in either diabetic or nondiabetic nerve. Similar results were obtained in the non–sodium-potassium–stimulated component (Fig. 1). Therefore, the statistically insignificant tendency for MI supplementation to raise diabetic composite ATPase activity reflected only the significant rise in the sodium-potassium ATPase component.

Relationship to homogenate protein concentration. When data were expressed per milligram Lowry protein instead of per gram wet weight of nerve, complete normalization of diabetic ouabain-inhibited and sodium-potassium stimulated ATPase activity was not achieved with dietary MI supplementation (data not shown). Instead, 1% dietary MI now raised diabetic sciatic nerve ouabain-inhibited and sodium-potassium–stimulated ATPase activity from 60% of normal to only 86% of normal, $P < 0.001$. However, this apparent shortfall in response derives entirely from an unexpected, small but statistically significant tendency for MI-treated diabetic nerve homogenates to have a

higher Lowry protein content (78.5±1.8 μg/mg wet weight in diabetics on the 1% MI diet vs. 72.0±1.4 μg/mg wet weight in diabetics on the standard diet [$P < 0.010$], 73.5±2.0 μg/mg wet weight in normal

FIGURE 1 Effect of experimental diabetes and 1% dietary MI supplementation on composite and sodium-potassium–stimulated ATPase activity in rat sciatic nerve. Sciatic nerve homogenates were prepared from nondiabetic rats, 4-wk diabetic rats, and 4-wk diabetic rats fed 1% MI diets, as described in Methods and Table II. ATPase activity was determined enzymatically by the method of Yoda and Yoda (22) in the presence and absence of sodium and potassium ions. Composite ATPase activity denotes the rate of ADP formation in the presence of both sodium and potassium ions, while sodium-potassium–stimulated ATPase activity (hatched bar) refers to the difference in the rate of ADP formation when sodium and potassium ions are omitted as discussed in Methods. Error bars indicate standard error of the mean for composite (upper error bar) and sodium-potassium–stimulated (lower error bar) ATPase activity. Figure is based on columns 1 and 3 of Table II.

rats on standard diet [P = NS], and 70.0±2.7 μg/mg wet weight in normal rats on the 1% MI diet [P < 0.025]). The basis for this anomaly is not understood, especially since nerve water content, usually reduced in our hands by acute experimental diabetes, was not affected by 1% dietary MI (18). This phenomenon is presently under investigation, and may possibly reflect an effect of dietary MI supplementation on axonal transport of proteins (8).

DISCUSSION

Acute streptozocin diabetes in the rat reduces sodium-potassium ATPase activity in crude homogenates of whole sciatic nerve by 40% (Fig. 1). Dietary MI supplementation, which prevents the characteristic fall in diabetic nerve MI (3, 7, 8), specifically and completely prevents the reduction in sodium-potassium ATPase activity, suggesting that the diabetic nerve sodium-potassium ATPase impairment results from MI deficiency. Furthermore, MI deficiency in diabetic peripheral nerve is caused by insulin deficiency and/or hyperglycemia (3), probably via competitive inhibition of sodium-dependent MI uptake by hyperglycemic concentrations of glucose (10) and/or increased polyol pathway activity (8, 11, 12). Thus, the sodium-potassium ATPase abnormality in diabetic peripheral nerve is an indirect consequence of insulin deficiency and/or its consequent hyperglycemia.

The sodium-potassium ATPase plays a central role in both nerve metabolism and function (24); accordingly, its 40% reduction in diabetes, if expressed in vivo, would appear to have important pathophysiological implications in experimental diabetic neuropathy. First, nerve conduction slowing in the spontaneously-diabetic BB rat has been attributed to a reduction in the resting transaxolemmal sodium electrochemical gradient (13) generated by the sodium-potassium ATPase (14). Second, nerve conduction slowing in the streptozocin diabetic rat is prevented by dietary MI administration (3), which normalizes nerve sodium-potassium ATPase activity. Thus, the conduction impairment in diabetic rat sciatic nerve and its correction by 1% dietary MI are probably both mediated by changes in sodium-potassium ATPase activity (18). (Peripheral nerve is a heterogeneous tissue comprised of axons, Schwann cells, the perineurial epithelium, and endo- and epineurial connective, vascular and adipose tissue, all of which may possess sodium-potassium ATPase activity. However, the electrophysiological observations cited above suggest that variation in whole-nerve homogenate sodium-potassium ATPase activity parallels that of the cellular elements responsible for nerve impulse conduction in vivo.)

The mechanism(s) relating intracellular free MI and sodium-potassium ATPase function are unclear. The strikingly high intracellular MI concentration characteristic of many mammalian cell lines has no well-defined biological role, although its major metabolic activity is reversible incorporation into membrane phosphoinositide (2, 25). Since tissue MI levels influence nondiabetic peripheral nerve phosphoinositide turnover (19), abnormalities in diabetic peripheral nerve phosphoinositide metabolism (26–28) may reflect altered tissue free MI levels (2). Since membrane phosphatidylinositol is an endogenous activator of renal microsomal sodium potassium ATPase in the rat (29), it is tempting to speculate that altered membrane phosphatidylinositol composition or metabolism underlies the sodium-potassium ATPase defect in diabetic rat peripheral nerve. The preservation of the sodium-potassium ATPase defect in cell-free homogenates of diabetic rat sciatic nerve is consistent with either a numerical reduction or compositional alteration in the sodium-potassium ATPase-membrane complex, the latter involving either the ATPase protein subunits themselves, their attached oligosaccharide residues, tightly-bound regulatory ligands, or the surrounding membrane lipid and protein milieu (30). Detailed metabolic and compositional studies in diabetic peripheral nerve are necessary to fully clarify the relationship(s) between nerve sodium-potassium ATPase activity and MI metabolism.

In summary. acute streptozocin diabetes reduces sciatic nerve MI concentration (3, 7, 8, 26, 27), conduction velocity (3, 6, 7) and sodium-potassium ATPase activity. MI administration obviates the fall in tissue MI and prevents the decrease in both nerve conduction velocity (3, 7, 8) and sodium-potassium ATPase. Since conduction impairment in experimental diabetes is attributable to a reduction in the ATPase-generated (14) sodium gradient (13), the ameliorative effect of MI administration on diabetic nerve conduction is likely mediated via a sodium-potassium ATPase effect. Because peripheral nerve sodium-potassium ATPase is fundamental to both energy (14) and substrate (31) metabolism, MI-related sodium-potassium ATPase perturbations might have widespread consequences in diabetic nerve (a recent report attributes the impaired "slow axonal transport" implicated in the "distal axonopathy" of diabetes [32], to altered diabetic rat nerve MI metabolism, since it is corrected by MI administration [8]). Finally, since nerve MI uptake is a sodium-gradient–dependent process (10), impaired sodium-potassium ATPase activity plus the competitive inhibition of sodium-dependent MI uptake by glucose (10) may synergistically blunt MI uptake by forming a self-reinforcing cycle of progressively reduced MI uptake, altered MI metabolism, and impaired sodium-

potassium ATPase function leading to widespread functional and possible structural alterations in diabetic peripheral nerve (18).

REFERENCES

1. Behse, F., F. Buchthal, and F. I. Carlsen. 1977. Nerve biopsy and conduction studies in diabetic neuropathy. *J. Neurol. Neurosurg. Psychiatry*. 40:1072–1082.
2. Clements, R. S. 1979. Diabetic neuropathy—new concepts of its etiology. *Diabetes*. 28:604–611.
3. Greene, D. A., P. V. DeJesus, Jr., and A. I. Winegrad. 1975. Effects of insulin and dietary myoinositol on impaired peripheral motor nerve conduction velocity in acute streptozocin diabetes. *J. Clin. Invest*. 55:1326–1336.
4. Eliasson, S. G. 1964. Nerve conduction changes in experimental diabetes. *J. Clin. Invest*. 43:2353–2358.
5. Winegrad, A. I., and D. A. Greene. 1976. Diabetic polyneuropathy: the importance of insulin deficiency, hyperglycemia and alterations in myoinositol metabolism in its pathogenesis. *N. Engl. J. Med*. 295:1416–1421.
6. 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.
7. Greene, D. A., R. A. Lewis, M. J. Brown, and S. A. Lattimer. 1982. Selective effects of *myo*-inositol administration on sciatic and tibial motor nerve conduction parameters in the streptozocin-diabetic rat. *Diabetes*. 31:573–578.
8. Mayer, J. H., and D. R. Tomlinson. 1983. The influence of aldose reductase inhibition and nerve *myo*-inositol on axonal transport and nerve conduction velocity in rats with experimental diabetes. *J. Physiol. (Lond.)*. In press.
9. Mayhew, J. A., K. R. W. Gillon, and J. N. Hawthorne. 1983. Free and lipid inositol, sorbitol and sugars in sciatic nerve obtained post-mortem from diabetic and control subjects. *Diabetologia*. 24:10–13.
10. Greene, D. A., and S. A. Lattimer. 1982. Sodium- and energy-dependent uptake of *myo*-inositol by rabbit peripheral nerve. *J. Clin. Invest*. 70:1009–1018.
11. Finegold, D., S. A. Lattimer, S. Nolle, M. Bernstein, and D. A. Greene. 1983. Polyol pathway activity and *myo*-inositol metabolism: a suggested relationship in the pathogenesis of diabetic neuropathy. *Diabetes*. In press.
12. Gillon, K. R. W., and J. N. Hawthorne. 1983. Sorbitol, inositol and nerve conduction in diabetes. *Life Sci*. 32:1943–1949.
13. Brismar, T., and A. A. F. Sima. 1981. Changes in nodal function in nerve fibres of the spontaneously diabetic BB-Wistar rat: potential clamp analysis. *Acta. Physiol. Scand*. 113:499–506.

14. Ritchie, J. M. 1967. The oxygen consumption of mammalian nonmyelinated nerve fibres at rest and during activity. *J. Physiol. (Lond.)*. 188:309–329.
15. Greene, D. A., and A. I. Winegrad. 1981. Effects of acute experimental diabetes on composite energy metabolism in peripheral nerve axons and Schwann cells. *Diabetes*. 30:967–974.
16. Das, P. K., G. M. Bray, A. J. Aguayo, and M. Rasminsky. 1976. Diminished ouabain-sensitive, sodium-potassium ATPase activity in sciatic nerves of rats with streptozotocin-induced diabetes. *Exp. Neurol*. 53:285–288.
17. Roelofsen, B. 1981. The (non)specificity in the lipid-requirement of calcium- and (sodium plus potassium)-transporting adenosine triphosphatases. *Life Sci*. 29:2235–2247.
18. Greene, D. A. 1983. Metabolic abnormalities in diabetic peripheral nerve: relationship to impaired function. *Metab. Clin. Exp*. 32(Suppl. 1):118–122.
19. Simmons, D. A., A. I. Winegrad, and D. B. Martin. 1982. Significance of tissue *myo*-inositol concentrations in metabolic regulation in nerve. *Science (Wash. DC)*. 217:848–851.
20. Ferrendelli, J. A., E. H. Rubin, H. T. Orr, D. A. Kinscherf, and O. H. Lowry. 1977. Measurement of cyclic nucleotides in histologically defined samples of brain and retina. *Anal. Biochem*. 78:252–259.
21. Lowry, O. H., N. J. Rosebrough, L. W. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem*. 193:265–275.
22. Yoda, A., and S. Yoda. 1980. A new simple method for NaK-ATPase rich membrane fragments. *Anal. Biochem*. 110:82–88.
23. Sokal, R. R., and F. J. Rohlf. 1969. Biometry: The Principles and Practice of Statistics in Biological Research. W. H. Freeman & Co., San Francisco.
24. Leone, J., and S. Ochs. 1978. Anoxic block and recovery of axoplasmic transport and electrical excitability of nerve. *J. Neurobiol*. 9:229–245.
25. Molitoris, B. A., I. E. Karl, and W. H. Daughaday. 1980. Concentration of myo-inositol in skeletal muscle of the rat occurs without active transport. *J. Clin. Invest*. 65:783–788.
26. Clements, R. S., and C. R. Stockard. 1980. Abnormal sciatic nerve *myo*-inositol metabolism in the streptozocin-diabetic rat. *Diabetes*. 29:227–235.
27. Palmano, K. P., P. H. Whiting, and J. N. Hawthorne. 1977. Free and lipid myoinositol in tissues from rats with acute and less severe streptozocin-induced diabetes. *Biochem J*. 167:229–235.
28. Natarajan, V., P. J. Dyck, and H. H. O. Schmid. 1981. Alterations of inositol lipid metabolism of rat sciatic nerve in streptozocin-induced diabetes. *J. Neurochem*. 36:413–419.
29. Mandersloot, J. G., B. Roelofsen, and J. De Gier. 1978. Phosphatidylinositol as the endogenous activatory of the ($Na^+ + K^+$)-ATPase in microsomes of the kidney. *Biochim. Biophys. Acta*. 508:478–485.
30. Trachtenberg, M. C., D. J. Packey, and T. Sweeney. 1981. In vivo functioning of the Na^+,K^+-activated ATPase. *In* Current Topics in Cellular Regulation. B. L. Horecker and E. R. Stadtman, editors. Academic Press, Inc. New York. 19:159–217.
31. Wheeler, D. D. 1975. Amino acid transport in peripheral nerve: specificity of uptake. *J. Neurochem*. 24:97–104.
32. Sidenius, P. 1982. The axonopathy of diabetic neuropathy. *Diabetes*. 31:356–364.