Transport of *myo*-inositol into endoneurial preparations of sciatic nerve from normal and streptozotocin-diabetic rats

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myo-Inositol transport by a viable rat sciatic-nerve preparation is described. Such 'endoneurial' nerve preparations accumulated *myo*-inositol by an energy-dependent saturable system. Streptozotocin-diabetes reduced *myo*-inositol transport into sciatic nerve by approx. 40%. Elevated medium glucose concentration reduced *myo*-inositol transport into control nerves to a similar extent. Fructose and sorbitol did not inhibit *myo*-inositol transport. Inclusion of an aldose reductase inhibitor in the medium counteracted the reduced *myo*-inositol transport caused by elevated glucose concentration. The importance of these results to the problem of diabetic neuropathy is discussed.

Abnormalities of the peripheral nervous system in human diabetes mellitus have been appreciated for many years (Mulder *et al.*, 1961). A reduction of motor-nerve conduction velocity (MNCV) also occurs in the rat on induction of experimental diabetes with streptozotocin (Sharma & Thomas, 1974; Greene *et al.*, 1975; Jakobsen, 1979; Brown *et al.*, 1980; Thomas *et al.*, 1981). It is now thought that this peripheral neuropathy in diabetes results from a metabolic abnormality. One such abnormality, decreased free *myo*-inositol concentration in diabetic rat sciatic nerve, has previously been reported (Greene *et al.*, 1975; Palmano *et al.*, 1977), and this reduction is consistent with reduced MNCV in acutely diabetic rats (Greene *et al.*, 1975). *myo*-Inositol is the precursor for the inositol-containing phospholipids, which are thought to play an important role in the propagation of nervous impulses (White & Larrabee, 1973; White *et al.*, 1974). Reduced concentration of total lipid *myo*-inositol in acutely diabetic rat nerve has been reported from this laboratory (Palmano *et al.*, 1977), as have alterations in the activities of some enzymes of inositol phospholipid metabolism (Whiting *et al.*, 1979). Decreased activity of CDP-diacylglycerol:*myo*-inositol transferase (Clements & Stockard, 1980) and abnormalities in labelling patterns of phosphoinositides (Hothersall & McLean, 1979; Clements & Stockard, 1980; Natarajan *et al.*, 1981; Bell *et al.*, 1982) in diabetic-rat nerve have also been reported.

One study of *myo*-inositol concentration in human nerve has so far been reported (Dyck *et al.*, 1980). No reduction was noted in nerve free *myo*-inositol

concentration in biopsied material from diabetic patients with clinical signs of neuropathy. However, we have recently completed a study of post-mortem sciatic nerve that shows a reduced concentration of free *myo*-inositol in nerves from diabetics compared with controls (Mayhew *et al.*, 1982).

myo-Inositol is maintained in peripheral nerve at a concentration that greatly exceeds that in serum (Greene *et al.*, 1975; Palmano *et al.*, 1977). Both synthesis from D-glucose and active accumulation could contribute to this. Synthesis of *myo*-inositol has been described for a number of tissues (Eisenberg, 1967; Whiting *et al.*, 1979), but only limited activity is present in rat sciatic nerve (Whiting *et al.*, 1979). It seems likely then that a mechanism for active accumulation of *myo*-inositol exists in peripheral nerve. Active transport of *myo*-inositol has been well characterized for a number of tissues (Hauser, 1965; Broeckhuyse, 1968; Caspary & Crane, 1970; Spector & Lorenzo, 1975; Gryan & Czech, 1979; Hammerman *et al.*, 1980), but there has, as yet, been no report of peripheral-nerve *myo*-inositol transport. The present study was designed to utilize a rat sciatic-nerve preparation *in vitro* to investigate the possibility of such a mechanism, and, if present, to observe the effects of experimental diabetes.

Experimental

Animals

Male Wistar rats were from the University of Nottingham Joint Animal Breeding Unit, Sutton Bonington, Leics., U.K., and received standard

Fig. 1. Diagrammatic representation of whole sciatic nerve (left) and fascicle preparation (right)

Removal of the perineurial membrane from the fascicle preparation gives the endoneurial preparation.

laboratory diet (Diet 41B; Wherry and Son, Bourne, Lincs., U.K.) *ad libitum*. All control rats were in the weight range 280–330 g. Diabetes was induced by a single intraperitoneal injection of streptozotocin (35 mg/kg body wt.) in 50 mM-sodium citrate buffer (pH 4.5). Control rats received a similar volume of buffer alone. Animals whose serum glucose concentration exceeded 16 mM 2 days after injection were included in the study. Diabetic animals and age-matched controls were used 16 weeks after injection.

Nerve dissection and preparation

Sciatic nerves were removed from the rats under pentobarbital (Sagatal; May and Baker, Dagenham, Essex, U.K.) anaesthesia (60 mg/kg body wt. by intraperitoneal injection). Two ligatures (with 5 'O'-grade silk) were applied to the nerve before removal, one at the sciatic notch and the other approx. 3 cm distal to the first.

Nerve preparation was performed by the method of Greene *et al.* (1979) for rabbit sciatic nerve, modified for the smaller rat nerve. After removal from the animal, the ligated nerve was placed in 2 ml of Krebs–Ringer bicarbonate buffer ('KRB buffer'; Krebs & Henseleit, 1932) containing 20 mM-glucose, 4% bovine serum albumin (fraction V; essentially fatty acid-free; Sigma London Chemical Co., Poole, Dorset, U.K.) and 7 mg of collagenase/ml (type I; Sigma) and incubated for 14 min at 37°C with a gas phase of O₂/CO₂ (19:1) on a shaking water bath. The contralateral nerve was removed from the animal during this 14 min incubation. Nerves were removed from the collagenase medium and placed in 8 ml of KRB buffer/bovine serum albumin/20 mM-glucose, containing aprotinin (200 kallikrein inhibitor units/ml of medium; Sigma), and the medium was bubbled with O₂/CO₂. Under a dissecting microscope and using watchmakers forceps, the epineurium was dissected away. The longest length of unbranched fascicle available was then ligated at each end (with 5 'O'-grade silk) and the original ligatures and

associated tissue were cut away. This resulted in the 'fascicle' preparation (Fig. 1).

To make the 'endoneurial' preparation, the 'fascicle' preparation was placed in 8 ml of KRB buffer/bovine serum albumin/5 mM-glucose, containing 50 μ M-*myo*-inositol. It was not found necessary to include *myo*-inositol at 500 μ M to maintain tissue *myo*-inositol during preparation and incubation as suggested by Greene *et al.* (1979). By using watchmakers forceps under a dissecting microscope the perineurial membrane was disrupted and removed from the length of the fascicle.

After a 5 min pre-incubation, the fascicle preparation was incubated in 2 ml of KRB buffer/bovine serum albumin/20 mM-glucose and the endoneurial preparation in KRB buffer/bovine serum albumin/5 mM-glucose/50 μ M-*myo*-inositol, on a shaking water bath with a gas phase of O₂/CO₂ (19:1). In experiments to study *myo*-inositol accumulation, 1 μ Ci of *myo*-[2-³H]inositol (sp. radioactivity 2–5 Ci/mmol) and 0.25 μ Ci of inulin[¹⁴C]carboxylic acid (sp. radioactivity 5–10 Ci/mol) (both obtained from Amersham International, Amersham, Bucks., U.K.) were added to the incubation medium.

Tissue preparation for analysis

For determination of ATP, phosphocreatine, glucose and *myo*-inositol, nerves were removed from the incubation medium, blotted dry and immediately frozen in liquid N₂. They were then powdered over 6% HClO₄ in a glass homogenizer with a glass pestle in a bath of liquid N₂ (Lowry & Passonneau, 1972). Homogenization was completed in thawing HClO₄ in an ethanol/ice bath at approx. –10°C (Lowry & Passonneau, 1972). Samples were centrifuged at 4°C and neutralized with KOH before storage at –80°C and analysis.

To determine tissue radioactivity after incubation, nerves were washed briefly in ice-cold incubation medium, blotted dry and immediately frozen and stored at –80°C. They were transferred to a freeze-drier with a shelf temperature of –40°C and freeze-dried to constant weight. Neutralized HClO₄ extracts of the tissue were made and, after centrifugation, portions of the supernatant were counted for radioactivity. Medium radioactivity was determined in zinc sulphate/barium hydroxide filtrates (Somogyi, 1945). To 0.5 ml samples of filtrates 0.5 ml of water and 10 ml of scintillation fluid (Fisofluor 1; Fisons, Loughborough, Leics., U.K.) were added for counting in a Packard 3375 liquid-scintillation counter. Correction for counting efficiency of ³H/¹⁴C dual label was achieved by using a Digital PDP 11/05 computer.

From the inulin radioactivity, the radioactivity (d.p.m.) per ml of intracellular fluid was calculated by the method of Fox *et al.* (1964). These values

were divided by the total medium radioactivity (d.p.m.) of *myo*-[2-³H]inositol and multiplied by the amount (nmol) of medium *myo*-inositol to obtain the amount (nmol) of medium *myo*-inositol appearing/ml of intracellular fluid.

Chemical determinations

ATP and phosphocreatine were determined by enzymic assay (Lowry & Passonneau, 1972) using hexokinase (yeast; 2mg/ml suspension), glucose 6-phosphate dehydrogenase (yeast; 5mg/ml suspension) and creatine kinase (rabbit muscle; all enzymes were from the Boehringer Corporation, London, U.K.) in an Aminco/Bowman Spectrophotofluorimeter.

Sorbitol was measured in neutralized HClO₄ extracts by fluorimetric assay using sorbitol dehydrogenase (sheep liver; Sigma) by the method of Clements *et al.* (1969).

Glucose and *myo*-inositol were determined in the neutralized HClO₄ extracts by g.l.c. as previously described (Palmano *et al.*, 1977). Serum glucose concentration was determined by using a Glucose Test-Kit (Boehringer, London, U.K.). Serum *myo*-inositol concentration was determined in barium hydroxide/zinc sulphate precipitates (Clements & Stockard, 1980). Portions of the supernatant obtained on centrifugation were subjected to g.l.c. as described previously (Palmano *et al.*, 1977).

Results

Animals

During experiments involving diabetic animals, control rats continued to gain weight, whereas diabetics lost, on average, 10% of their original weight (Table 1).

Serum glucose was significantly elevated in diabetic animals but diabetes did not induce a significant change in serum *myo*-inositol concentration (Table 1).

Nerve preparations

To check the viability of the nerve preparations, some of the estimations suggested by Greene *et al.* (1979) were made. High-energy phosphate compounds and glucose concentrations were determined before and after incubation of fascicle and endoneurial preparations in their respective media.

No significant changes occurred in fascicle ATP, phosphocreatine or glucose concentrations after a 60min incubation, although ATP concentration increased (Table 2). Phosphocreatine concentrations in the endoneurial preparation were found to be significantly decreased ($P < 0.05$) compared with those of the fascicle preparation, but remained stable during 60min of incubation. ATP and glucose concentrations rose slightly, but insignificantly, during the incubation (Table 2).

Table 1. *Body weight, serum glucose and myo-inositol concentrations of control and diabetic rats*

Results are expressed as means ($\pm$ S.E.M.) for the numbers of animals shown in parentheses. Significance limits between control and diabetic groups were determined by Student's *t* test. *, $P < 0.001$.

	Control	Diabetic
Body wt. (g)		
Initial	313 $\pm$ 5 (10)	318 $\pm$ 9 (10)
Final	401 $\pm$ 7 (10)	286 $\pm$ 12 (10)*
Serum glucose (mM)	8.9 $\pm$ 0.8 (9)	32.5 $\pm$ 2.0 (9)*
Serum <i>myo</i> -inositol (μ M)	81.8 $\pm$ 4.92 (10)	73.6 $\pm$ 4.62 (8)

Table 2. *ATP, phosphocreatine, glucose and myo-inositol concentrations of fascicle and endoneurial preparations of rat sciatic nerve*

Results are expressed as means ($\pm$ S.E.M.) in μ mol/g wet wt. of tissue. The numbers of animals are shown in parentheses. Significance limits were determined by Student's *t* test. *, $P < 0.001$.

	Zero min	60 min
Fascicle		
ATP	0.49 $\pm$ 0.02 (5)	0.57 $\pm$ 0.08 (4)
Phosphocreatine	1.64 $\pm$ 0.16 (5)	1.57 $\pm$ 0.15 (4)
Glucose	1.96 $\pm$ 0.31 (5)	2.17 $\pm$ 0.56 (5)
<i>myo</i> -Inositol	3.68 $\pm$ 0.14 (4)	3.02 $\pm$ 0.30 (4)
Endoneurial		
ATP	0.56 $\pm$ 0.11 (3)	0.63 $\pm$ 0.07 (4)
Phosphocreatine	0.93 $\pm$ 0.12 (3)	1.08 $\pm$ 0.23 (4)
Glucose	1.81 $\pm$ 0.15 (4)	2.24 $\pm$ 0.33 (4)
<i>myo</i> -Inositol	3.90 $\pm$ 0.09 (4)	4.66 $\pm$ 0.07 (4)*

Clearly, the ability of the preparations to maintain *myo*-inositol concentration during incubation is important in a study of the uptake mechanisms of the compound. During a 60min incubation in the absence of medium *myo*-inositol, the fascicle *myo*-inositol concentration decreased, though not significantly. The endoneurial preparation, in contrast, not only maintained *myo*-inositol concentration for 60min of incubation, but also accumulated *myo*-inositol from the medium to increase tissue concentration by 18%. This contrasts with the findings of Greene *et al.* (1979), who showed that rabbit endoneurial preparations required the presence of 500 μ M medium *myo*-inositol to maintain nerve *myo*-inositol concentrations over 120min of incubation.

myo-Inositol accumulation

After a 60min incubation with *myo*-[2-³H]inositol without carrier, the fascicle preparation did not appear to actively accumulate *myo*-inositol from the medium, since the tissue/medium ratio of radioactivity did not exceed unity [0.63 ± 0.20 (mean $\pm$ S.E.M.; $n = 4$)]. However, incubation of the

Fig. 2. Effect of medium myo-inositol concentration on uptake by rat sciatic-nerve endoneurial preparation *in vitro*

Each point represents the mean of results from the number of rats indicated. Bars indicate $\pm$ s.e.m.

endoneurial preparation with labelled myo-inositol in carrier-free medium resulted in a ratio $[4.52 \pm 0.55$ ($n=4$)] that suggested that a mechanism for concentration of myo-inositol was present. It appears that dissection of the perineurial membrane removes a barrier to the transport of myo-inositol in peripheral nerve. For this reason, the endoneurial preparation was used for all subsequent myo-inositol accumulation studies.

Time course

The accumulation of myo-inositol from the standard medium into intracellular fluid by the endoneurial preparation proceeded at a constant rate of 2.0 nmol/ml of intracellular fluid per min throughout 60 min of incubation.

Effect of medium myo-inositol concentration

Increasing medium myo-inositol concentration resulted in a reduction in the rate of increase of accumulation of myo-inositol by the endoneurial preparation, suggestive of a saturable transport system (Fig. 2). A plot of the 60 min data was made by the method of Eisenthal & Cornish-Bowden (1974). This revealed a K_m for myo-inositol transport of 225 μ M and a V_{max} of 9.02 nmol of myo-inositol accumulated/ml of intracellular fluid per min. The process appears to be an energy-dependent one linked to $(Na^+ + K^+)$ -dependent ATPase activity, since inclusion of 2 mM-ouabain in the medium inhibited myo-inositol accumulation by 50% (Table 3).

Table 3. Accumulation of myo-[2- 3H]inositol by rat sciatic-nerve endoneurial preparation: effect of diabetes and medium composition

Results are means ($\pm$ s.e.m.), for the numbers of animals shown in parentheses. Significance limits were determined by Student's *t* test. †, $P < 0.001$, versus age-matched control; *, $P < 0.01$, versus non-injected weight-matched controls; **, $P < 0.001$, versus non-injected weight-matched controls; ††, $P < 0.01$, versus '10 μ M-Sorbinil' and versus '30 mM-glucose'.

	myo-Inositol accumulation (nmol/ml of intracellular fluid per h)
Age-matched control	117.0 $\pm$ 8.09 (10)
Diabetic	73.1 $\pm$ 4.20 (10)†
Non-injected weight-matched control	123.3 $\pm$ 9.45 (6)
Change in medium composition	
2 mM-Ouabain	61.0 $\pm$ 4.82 (5)**
30 mM-Glucose	77.6 $\pm$ 3.24 (6)*
30 mM-Fructose	117.9 $\pm$ 5.18 (3)
30 mM-Sorbitol	156.8 $\pm$ 18.41 (3)
30 mM-Glucose + 10 μ M-Sorbinil	99.6 $\pm$ 1.78 (3)††
10 μ M-Sorbinil	136.2 $\pm$ 6.40 (3)

myo-Inositol accumulation and diabetes

Since myo-inositol appeared to be concentrated by peripheral nerve by an energy-dependent saturable system, the effect on the system of the metabolic changes that occur during diabetes was investigated. Endoneurial preparations of sciatic nerves of age-matched control rats accumulated myo-inositol from the standard medium into intracellular fluid at approx. 120 nmol/ml of intracellular fluid per h, corresponding to a tissue/medium radioactivity ratio of approx. 2.5. Diabetes of 4 months duration resulted in a 40% reduction in this rate (Table 3). Each observation is presented as the mean for duplicate nerves from each animal. We believe no bias was introduced by this method since the results for nerves from right and left legs did not differ significantly (e.g. for age-matched control animals: animal mean, 117.0 $\pm$ 8.09; left-leg nerves, 118.4 $\pm$ 6.60; right-leg nerves, 115.5 $\pm$ 12.16 nmol/ml of intracellular fluid per h).

Nerves from control animals weighing approx. 300 g accumulated myo-inositol at a similar rate compared with nerves from age-matched controls (Table 3). Therefore, by additions to the medium, it was possible to investigate factors that might affect the transport during experimental diabetes. Elevated glucose concentrations have been shown to inhibit myo-inositol transport *in vitro* in other tissues (Hauser, 1965; Broeckhuysen, 1968; Caspary &

Table 4. *Glucose, sorbitol and myo-inositol concentrations in sciatic-nerve endoneurial preparations after incubation*
Results are means ($\pm$ s.e.m.) in $\mu\text{mol/g}$ wet wt. of tissue for the numbers of animals given in parentheses. Significance limits were determined by Student's *t* test. *, $P < 0.02$, versus control; **, $P < 0.01$, versus control; ***, $P < 0.001$, versus control; †, $P < 0.01$, versus '30 mM-glucose'.

	Glucose	Sorbitol	myo-Inositol
Control	2.24 ± 0.33 (4)	0.13 ± 0.04 (8)	4.66 ± 0.07 (4)
Diabetic	3.91 ± 0.35 (4)*	1.78 ± 0.22 (4)***	2.75 ± 0.09 (4)***
Change in medium composition			
30 mM-Glucose	9.29 ± 2.03 (3)**	0.37 ± 0.04 (4)***	—
30 mM-Glucose + 10 μM -Sorbitinil	—	0.11 ± 0.02 (3)†	—

Crane, 1970; Hammerman *et al.*, 1980). Since increased serum glucose concentration during experimental diabetes also results in increased nerve glucose concentrations (Greene *et al.*, 1975; Palmano *et al.*, 1977) endoneurial preparations were incubated in media with 30 mM-glucose. This resulted in an approx. 40% reduction of the rate of *myo*-inositol accumulation (Table 3). This inhibition is relatively specific, since medium containing 30 mM-fructose or 30 mM-sorbitol did not induce a significant decrease (Table 3). Indeed, *myo*-inositol accumulation in the presence of 30 mM-sorbitol increased by approx. 25%.

When nerves were incubated in medium containing 30 mM-glucose with the addition of an aldose reductase inhibitor at 10 μM (CP-45,634; Sorbinil; Pfizer), a significant increase in the rate of *myo*-inositol accumulation compared with nerves incubated with 30 mM-glucose alone was observed (Table 3). This increased rate was also significantly lower than that obtained when nerves were incubated with only the aldose reductase inhibitor at 10 μM in standard medium containing 5 mM-glucose. Although the concentration of glucose in the nerves from diabetic animals fell during incubation compared with non-incubated nerves (Table 4), the increased sorbitol concentration observed in diabetic rat nerves (Palmano *et al.*, 1977) persisted. Incubation of nerves from control animals in medium containing 30 mM-glucose resulted in nerve glucose concentrations similar to those observed in diabetic-rat non-incubated nerve and sorbitol values approx. 3-fold higher than control values. The addition of aldose reductase inhibitor to the medium prevented this increase in nerve sorbitol concentration (Table 4).

Discussion

The development of a metabolically stable preparation of rabbit peripheral nerve that overcame the problems of membrane barriers to the external medium was important to the study of the biochemistry of isolated nerve (Greene & Winegrad, 1979). In the present paper a modification of this

technique was used to study some aspects of rat peripheral-nerve *myo*-inositol biochemistry. The rat sciatic fascicle and endoneurial preparations also maintained ATP, phosphocreatine and glucose concentrations during preparation and incubation and seemed suitable for further research. The high concentrations of *myo*-inositol found in peripheral nerve were also maintained during preparation and incubation and could even be increased by medium supplementation with *myo*-inositol.

It was immediately evident from the transport experiments that no mechanism for the active translocation of *myo*-inositol through the perineurial membrane exists, since the tissue/medium ratio of inositol radioactivity in the fascicle preparation did not exceed unity. This, and the demonstration that the fascicle-preparation *myo*-inositol concentration did not decrease significantly during incubation for 1 h, suggests that impermeability of the perineurial membrane to *myo*-inositol is the means whereby *myo*-inositol concentration within the endoneurium is maintained. In contrast, the 7-fold increase in the tissue/medium radioactivity ratio observed when the endoneurial preparation was incubated with tracer *myo*-inositol indicates that *myo*-inositol in the extracellular space of the endoneurium is actively removed into cellular elements. This process appears to be a saturable one (Fig. 2). A saturable *myo*-inositol transport system is found in other tissues (Caspary & Crane, 1970; Spector & Lorenzo, 1975; Gryan & Czech, 1979; Hammerman *et al.*, 1980), though not in synaptosomes (Warfield *et al.*, 1978). Also demonstrated in other tissues is the energy-dependence of the process (Hauser, 1965; Broeckhuysen, 1968; Caspary & Crane, 1970; Gryan & Czech, 1979), and sciatic nerve is no exception, accumulation of *myo*-inositol into intracellular fluid being sensitive to inhibition of ($\text{Na}^+ + \text{K}^+$)-stimulated ATPase activity with ouabain (Table 3).

The decreased rate of *myo*-inositol accumulation by peripheral nerves from diabetic animals is clearly consistent with the lowered concentration in such nerves. Decreased transport of *myo*-inositol into

diabetic-rat nerve has previously been suggested, since experiments *in vivo* indicated a diminished space of distribution of *myo*-[2-³H]inositol (Clements & Stockard, 1980), and experiments *in vitro* demonstrated decreased incorporation of *myo*-inositol into phosphatidylinositol in diabetic-rat nerves, which was increased in broken-cell preparations (Hothersall & McLean, 1979). An attractive possibility for this defect is an effect on *myo*-inositol transport of elevated ambient glucose concentrations during diabetes, since inhibition of *myo*-inositol transport *in vitro* by glucose has been shown in other tissues (Hauser, 1965; Broeckhuyse, 1968; Caspary & Crane, 1970; Hammerman *et al.*, 1980). Incubation of the endoneurial preparation in 30 mM-glucose medium inhibited *myo*-inositol accumulation by approx. 37%. The elevation of nerve glucose (by some 400%) during this incubation is consistent with the inhibition of *myo*-inositol transport into intracellular fluid. However, after incubation of the diabetic-rat nerves in 5 mM-glucose medium, the glucose concentration in the nerve was only 74% higher than that in controls instead of the 4–6-fold increase normally seen in diabetic-rat nerves. The high concentration of sorbitol in the nerves from diabetic animals persisted during incubation, suggesting that sorbitol does not readily diffuse from cellular elements of peripheral nerve. Incubation of nerves in 30 mM-glucose medium also resulted in elevated nerve sorbitol concentrations, though these were much lower than in nerves of diabetic animals. The increase in sorbitol levels during incubation with 30 mM-glucose could be prevented by inclusion of an aldose reductase inhibitor in the medium, and this resulted in a significant improvement in the rate of *myo*-inositol accumulation by nerves incubated in 30 mM-glucose. It is possible, therefore, that a combination of increased nerve glucose and sorbitol concentrations is responsible for the defect in *myo*-inositol transport in nerves from diabetic rats, though added external sorbitol increases the rate of transport (Table 3). Much of the free *myo*-inositol in peripheral nerve is thought to be located in Schwann cells (Kusama & Stewart, 1970), also a proposed site of aldose reductase activity (Gabbay & O'Sullivan, 1968; Ludvigson & Sorenson, 1980). Perhaps elevated Schwann-cell sorbitol concentrations during diabetes affect the ability of the cell to concentrate and maintain *myo*-inositol. Indeed, it has been suggested that accumulation of galactitol in sciatic nerve during galactose feeding results in osmotic changes and lowered *myo*-inositol concentration without direct inhibition of *myo*-inositol synthesis or uptake (Stewart *et al.*, 1968). Our experiments do not exclude the possibility that a membrane defect caused by the diabetic state, or directly by streptozotocin, damages the inositol transport system.

The present study has not attempted to determine whether the effectiveness of the perineurial membrane as a barrier to the release of endoneurial compounds is affected by diabetes. One study has shown that leakage of *myo*-inositol from the lens *in vitro* is increased in diabetes (Wada *et al.*, 1979). A study of release of preloaded labelled *myo*-inositol from control and diabetic-rat nerves *in vitro* may therefore prove useful.

We believe that these results have important implications for peripheral nerve function during diabetes. It is proposed that one factor in the aetiology of diabetic neuropathy may be the reduced *myo*-inositol accumulation by peripheral nerve and that this is responsible for altered inositol phospholipid metabolism.

The demonstration of a modifiable *myo*-inositol transport mechanism in peripheral nerve allows steps to be taken to normalize nerve concentrations *in vivo*. Dietary supplementation in rats has been shown to be successful in this regard (Greene *et al.*, 1975; Jefferys *et al.*, 1978). In the light of the effect of the aldose reductase inhibitor on *myo*-inositol concentration reported here, a further study of the effects of this compound *in vivo* is desirable.

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