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Free and Lipid *myo*-Inositol in Tissues from Rats with Acute and Less Severe Streptozotocin-Induced Diabetes

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Acute diabetes with ketosis was induced in rats by intraperitoneal streptozotocin, and also a milder form of diabetes without ketosis by injecting less of the drug. The acutely diabetic rats were killed 72h after injection and the others after either 2 or 13 weeks. Free and lipid *myo*-inositol was then measured in various tissues and body fluids by g.l.c. of the trimethylsilyl ether. Serum inositol was increased in the acutely diabetic group, whereas liver inositol was decreased. Brain and kidney inositol concentrations were increased in the mildly diabetic animals at 13 weeks and there was a progressive decrease in sciatic-nerve inositol. Lipid inositol of sciatic nerve was decreased in the acutely diabetic group only. Brain lipid inositol concentration was decreased in mild diabetes at 13 weeks. Possible implications of these findings in relation to diabetic neuropathy are discussed.

There have been numerous reports on the involvement of hexitols and related compounds in lesions of the peripheral nervous system. In particular, it has been shown that a decrease in inositol content in sciatic nerve of rats with experimental diabetes accompanies decreased motor-nerve conduction velocity (Greene *et al.*, 1975). In conjunction with this, the above workers have reported that inositol feeding can prevent the decrease in both inositol content and conduction velocity, despite persistent hyperglycaemia and elevated nerve sorbitol and fructose concentrations. The relationship between inositol metabolism and nerve conduction velocity is not yet understood, but it is possible that the inositol-containing lipids are also involved. These phospholipids have a rapid turnover in nervous tissue, and diphosphoinositide (1-phosphatidylinositol 4-phosphate) and triphosphoinositide (1-phosphatidylinositol 4,5-bisphosphate) are particularly associated with myelin (Eichberg & Dawson, 1965; Hawthorne & Kai, 1970). Furthermore triphosphoinositide may be metabolically involved in axonal conduction (White & Larrabee, 1973; White *et al.*, 1974). The neuropathy associated with diabetes mellitus involves segmental demyelination and axonal damage (Thomas & Lascelles, 1966; Chopra *et al.*, 1969) and abnormalities of lipid metabolism have been demonstrated in peripheral nerves in experimental diabetes (Eliasson, 1964, 1966; Eliasson & Samet, 1969).

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To throw some light on the possible involvement of inositol and inositol-containing lipids in diabetic neuropathy, a comparative study of these compounds in various tissues of rats with experimentally induced diabetes was undertaken. The study was designed so that any changes occurring might be correlated with either severity or duration of the diabetes.

Materials and Methods

Special chemicals

Streptozotocin was a gift of Upjohn Co., Kalamazoo, MI, U.S.A. For g.l.c., 3% SE-30 on 80/100 Supelcoport was obtained from Supelco, Bellefonte, PA, U.S.A. Pyridine, from BDH Chemicals, Poole, Dorset, U.K., was redistilled before use and stored in a desiccator over $CaCl_2$ pellets. Amberlite MB 3 ion-exchange resin was also obtained from BDH. Methyl α-D-mannopyranoside was from Koch–Light Laboratories, Colnbrook, Bucks., U.K. Urease (type III), from Sigma Chemical Co., St. Louis, MO, U.S.A., was stored desiccated at 4°C. Clinistix were purchased from Ames Co., Stoke Poges, Bucks., U.K., and the Biochemica Test Combination for blood sugar was from Boehringer Mannheim G.m.b.H., Mannheim, W. Germany. Insulin RIA Kit IM.78 was supplied by The Radiochemical Centre, Amersham, Bucks., U.K. All other chemicals were of analytical grade.

Animals

Male Wistar rats (250–350g) were from the University of Nottingham Joint Animal Breeding

Unit, Sutton Bonington, Leics., U.K., and were maintained on standard laboratory diet (Diet 41B; Oxoid, London SE1 9HF, U.K.) in metabolic cages (Jencons, Hemel Hempstead, Herts., U.K.). They were fed and watered *ad libitum* throughout the study. Age and weight-matched animals were divided into experimental and control groups.

Induction of diabetes

For the purposes of this study, it was decided to use two models of experimental diabetes, an 'acute ketoacidotic' form (group 1) and a 'mild chronic' form, the latter being subdivided into a relatively short-term (group 2) and a long-term diabetes (group 3). Diabetes was induced by intraperitoneal injection of streptozotocin in approx. 0.2ml of 50mM-sodium citrate buffer, pH4.5, at a dosage of 75mg/kg body wt. for group 1 and 35mg/kg body wt. for groups 2 and 3. Diabetes was established within 24h as judged by the classical criteria of hyperglycaemia and glycosuria. Those animals that excreted less than 1g of glucose within 24h (Clinistix) were discarded. Body weight, metabolites in blood and urine, and food and water intake were monitored. The group-1 rats became acutely diabetic within 24h and were killed 72h after injection of streptozotocin. Those rats on a lower dosage of streptozotocin (groups 2 and 3) were killed at 2 and 13 weeks respectively. Control rats were killed 2 weeks after initiation of the study.

A symptomatic and biochemical classification of the diabetic syndrome, according to several criteria, is given for each group in Table 1. Control values are also included. The data show that each of the diabetic groups is clearly differentiated from the controls, and that the 'acute' form of diabetes can be distinguished from the milder forms on the basis of extreme liver glycogen depletion and severe ketoacidosis. Biochemical differences between groups 2 and 3 are fewer and less striking, but there is gradual depletion of glycogen stores, and serum non-esterified fatty acids increase. However, a gross deterioration in physical condition is observed in the 13-week-diabetic group.

Assays

Glucose in blood and urine was assayed by the method of Werner *et al.* (1970) by using a Boehringer glucose oxidase kit. Insulin was measured by radioimmunoassay (see under 'Special chemicals'). Glycogen was determined by the method of Good *et al.* (1933). Ketone bodies were assayed by the method of Williamson *et al.* (1962), and non-esterified fatty acids by the method of Elphick (1975).

Preparation of tissue extracts

Dissections were carried out at 4°C. Rats were killed by cervical dislocation and decapitation. Blood was collected from the neck, allowed to clot for 30min at 4°C, centrifuged (800*g* for 15 min) and the supernatant serum drawn off and frozen rapidly. Sciatic nerves, kidney and liver tissue were dissected out, cleaned of adherent blood vessels and connec-

Table 1. *General features of 'acute' and 'mild chronic' diabetes in streptozotocin rats as compared with controls*
Biochemical data are presented as means±S.D. or as ranges for the numbers of determinations in parentheses. ND, not detectable.

			Long-term mild diabetes	
	Control	Acute diabetes Group 1	Group 2	Group 3
Streptozotocin dosage (mg/kg)	—	75	35	35
Duration of diabetes	—	72h	2 weeks	13 weeks
Weight at death (g)	260±7.9 (10)	213±10.2 (8)	223±4.9 (10)	215±8.3 (10)
Weight loss (%)	—	13.4±2.9 (8)	19.3±2.5 (10)	17.7±5.0 (10)
Serum insulin (μ-i.u./ml)	23.5±4.6 (10)	ND	ND	ND
Serum non-esterified fatty acids (mM)	0.33±0.04 (5)	2.32±1.1 (5)	0.45±0.1 (5)	0.80±0.29 (10)
Serum ketone bodies, total (mM)	0.16±0.04 (5)	28.4±3.32	0.42±0.12 (5)	0.62±0.10 (5)
Serum glucose (mM)	8.19±0.39 (5)	103±28 (5)	37.9±4.4 (5)	24.8±5.8 (9)
Urine glucose (mg/24h)	0.2–2.00 (10)	1114–4045 (8)	1970–4717 (10)	3000–10000 (10)
Liver glycogen (μmol/g wet wt.)	302±31.9 (5)	0.33±0.16 (5)	128±18 (5)	78±22 (5)
Food and water intake	Normal	Decreased	Increased	Increased
Physical condition	Normal	Very lethargic and weak; dehydrated	Near normal activity; some evidence of fat store depletion and muscle wasting; fatty liver	Lethargic and weak; gross depletion of fat stores around organs; muscle wasting; evidence of cataract formation and organ lesions

tive tissue and frozen immediately in liquid N_2. Tissues were stored at −20°C until extraction. Whole brain was dissected out, cleaned, weighed and processed immediately.

Extraction of water-soluble and lipid fractions from tissues

(*a*) *Brain, kidney and liver tissues.* Weighed samples (approx. 1.0–1.5g wet wt.) were homogenized in 10vol. of ice-cold 0.33M-$HClO_4$ and the supernatant was separated by centrifugation at 2000*g* for 10min. The precipitate was re-extracted with 6vol. of ice-cold 0.33M-$HClO_4$. Duplicate 1ml samples of the combined $HClO_4$ extracts were neutralized with saturated $KHCO_3$ solution. The $KClO_4$ precipitate was removed by centrifugation (1500*g* for 10min) and the supernatants were deionized by passage through columns (1cm×4cm) of Amberlite MB 3 mixed-bed ion-exchange resin. The columns were washed with 3 successive bed volumes of deionized water, the eluates collected and freeze-dried with internal standard in preparation for measurement of free sugars by g.l.c.

The precipitate was subjected to lipid extraction by the acidified chloroform/methanol method of Wells & Dittmer (1965), modified by use of 1M-HCl containing 30mM-$CaCl_2$ at phase separation, as recommended by Dawson (1965). Phases were separated by centrifugation. The washed lower organic phase was evaporated to dryness by rotary evaporation at 30°C, the lipid film was redissolved in 5ml of chloroform/methanol (1:1, v/v) and samples (1ml) were taken for acid hydrolysis.

(*b*) *Sciatic nerve.* Weighed frozen tissue (approx. 50mg wet wt.) was homogenized by hand in 2ml of ice-cold 0.33M-$HClO_4$ in a ground-glass pestle and mortar. The mortar was washed with 1ml of 0.33M-$HClO_4$ and the wash combined with the homogenate. The precipitate from centrifugation was re-extracted with 2ml of 0.33M-$HClO_4$ and samples (1ml) of the combined supernatants were treated for g.l.c. as described below, after neutralization and deionization as for brain and other tissues.

The lipids in the $HClO_4$ precipitates were extracted by the method of Wells & Dittmer (1965) by using 3×2ml of chloroform / methanol / conc. HCl (500:500:3, by vol.) and the phases separated by addition of 0.2vol. of 1M-HCl containing 30mM-$CaCl_2$. The washed lower phases were evaporated to dryness under a stream of N_2, redissolved in 3ml of chloroform/methanol (1:1, v/v) and 1ml samples taken for acid hydrolysis.

Acid hydrolysis to release inositol from lipid

Portions of lipid extracts were taken to dryness in glass ampoules and 1ml of 6M-HCl was added. The ampoules were sealed, then heated at 120°C for 20h. After cooling, the ampoules were opened and HCl was removed by vacuum desiccation over NaOH at 55°C for 2h. The lipid hydrolysates were redissolved in 1ml of water and passed through columns (1cm×4cm) of Amberlite MB 3 to deionize. The eluates from this and two successive column washes were collected into tubes containing 10μg of methyl α-D-mannopyranoside (the g.l.c. internal standard) and the contents were freeze-dried.

Gas–liquid chromatography

Inositol and free sugars in freeze-dried tissue extracts and hydrolysates were converted into their trimethylsilyl ethers (Sweeley *et al.*, 1963) by reaction with 100μl of pyridine/hexamethyldisilazane/trimethylchlorosilane (5:2:1, by vol.). The tubes were mechanically shaken for 4h to ensure complete reaction. Samples were chromatographed on a Pye series 104 gas–liquid chromatogram equipped with hydrogen flame-ionization detectors. Portions (1–2μl) of the reaction mixture were injected with a 10μl micro-syringe on to a 180cm glass column packed with 3% SE-30 on Supelcoport, and chromatographed with N_2 as carrier gas (flow rate 35ml/min) and an oven-temperature program of 180°C for 6min followed by an increase of 3°C/min to 200°C. Sugars were identified by comparison of retention times with those of known standards, and quantification was effected by relating peak area of sugar (peak height × width at ½ peak height) to that of internal standard. Calibration curves were prepared periodically.

Preparation of serum and urine for analysis of free sugars

Analyses were carried out in duplicate.

(*a*) *Serum.* Samples of thawed serum (0.5ml) were deproteinized by the method of Somogyi (1945) and the deproteinized supernatants were freeze-dried with 10μg of methyl α-D-mannopyranoside, in preparation for g.l.c. The dried samples were trimethylsilylated and chromatographed as described above.

(*b*) *Urine.* Samples (1ml) of 24h urine specimens were prepared for g.l.c. essentially as described by Wells *et al.* (1964) by using urease type III (Sigma) and Amberlite MB-3 mixed-bed ion-exchange resin. Freeze-dried urine extracts were treated with silylating reagent and chromatographed as above.

Results

Sugars in the $HClO_4$ supernatants

The concentrations of free sugars in tissue extracts of control and experimental animals are shown in

Table 2. *Free sugars and polyols in tissue extracts of control and diabetic rats*

Free sugars in $HClO_4$ extracts and biological fluids were assayed by g.l.c. as described in the Materials and Methods section. Table 1 gives details of treatment for each group. Results show mean values ± S.D. for each group, and numbers of determinations are given in parentheses. Significance limits between control and diabetic groups, as determined by Student's *t* test, are given with the results; significance of difference within diabetic groups is given separately within the Table. *$P<0.05$; †$P<0.01$; NS, not significant ($P>0.1$).

		Content (μmol/g wet wt.)				Differences between diabetic groups		
			Diabetic					
		Control	Group 1	Group 2	Group 3	1 vs. 2	1 vs. 3	2 vs. 3
Inositol	Serum§	0.08 ± 0.01 (9)	0.17 ± 0.09 (7)*	0.07 ± 0.02 (4)	0.08 ± 0.02 (9)	*	†	NS
	Sciatic nerve	3.10 ± 0.34 (10)	3.02 ± 0.43 (8)	2.66 ± 0.47 (11)*	2.44 ± 0.48 (9)†	*	†	NS
	Brain	6.62 ± 0.96 (14)	5.84 ± 0.87 (8)	6.28 ± 1.01 (10)	8.3 ± 1.60 (9)†	NS	†	†
	Liver	0.57 ± 0.12 (10)	0.32 ± 0.08 (8)†	0.53 ± 0.16 (10)	0.58 ± 0.11 (9)	†	†	NS
	Kidney	4.51 ± 0.36 (5)	3.80 ± 1.20 (8)	—	5.60 ± 0.45 (9)†	—	†	—
	Urine‡	112 ± 74 (13)	74 ± 78 (8)	195 ± 201 (14)	194 ± 124 (9)*	NS	*	NS
Glucose	Serum§	5.88 ± 0.64 (10)	24.2 ± 6.1 (7)†	11.4 ± 7.2 (7)†	17.9 ± 5.2 (18)†	*	*	†
	Sciatic nerve	2.03 ± 0.40 (10)	10.12 ± 3.96 (8)†	7.94 ± 2.62 (11)†	13.3 ± 4.17 (9)†	NS	NS	†
	Brain	0.59 ± 0.19 (10)	4.72 ± 2.30 (8)†	1.41 ± 1.22 (10)*	2.29 ± 1.14 (9)†	†	†	NS
	Liver	9.14 ± 7.06 (6)	15.97 ± 8.88 (8)	28.5 ± 11.7 (5)†	31.3 ± 6.11 (9)†	*	†	NS
	Kidney	1.57 ± 0.29 (5)	18.0 ± 7.85 (8)†	—	24.1 ± 7.4 (9)†	—	NS	—
	Urine‡	5.2 ± 5.9 (13)	2197 ± 1073 (8)	3525 ± 3417 (14)	3451 ± 2973 (9)†	NS	NS	NS
Sorbitol	Serum§	0.007 ± 0.003 (5)	0.035 ± 0.04 (7)	—	0.025 ± 0.01 (9)†	—	NS	—
	Sciatic nerve	0.14 ± 0.09 (10)	0.41 ± 0.04 (8)†	0.57 ± 0.44 (11)†	0.89 ± 0.53 (9)†	NS	†	NS
	Brain	0.008 ± 0.002 (5)	0.126 ± 0.04 (8)†	0.10 ± 0.02 (4)†	0.09 ± 0.03 (9)†	NS	*	NS
	Liver	0.16 ± 0.12 (5)	0.017 ± 0.006 (8)†	0.08 ± 0.08 (4)	0.03 ± 0.03 (9)†	*	NS	NS
	Kidney	0.12 ± 0.03 (5)	0.18 ± 0.06 (8)*	—	0.30 ± 0.15 (9)*	—	*	—
Fructose	Serum§	0.18 ± 0.09 (9)	2.20 ± 1.95 (7)†	0.04 ± 0.02 (4)†	0.33 ± 0.08 (9)†	†	†	†
	Sciatic nerve	0.30 ± 0.09 (10)	2.56 ± 0.62 (8)†	2.70 ± .163 (11)†	2.63 ± 0.87 (9)†	NS	NS	NS
	Brain	0.05 ± 0.03 (9)	0.74 ± 0.41 (8)†	0.12 ± 0.07 (10)†	0.20 ± 0.09 (9)†	†	†	*
	Liver	0.25 ± 0.14 (8)	1.02 ± 0.63 (8)†	0.42 ± 0.33 (10)	0.77 ± 0.15 (9)†	†	NS	†
	Kidney	0.19 ± 0.03 (5)	1.43 ± 0.54 (8)†	—	0.55 ± 0.21 (9)†	—	*	—

‡ Urine values expressed as mg excreted/24h.
§ Serum values expressed as μmol/ml.

Table 2. In all three groups of diabetic rats glucose in the various tissues is increased well above the normal concentration. It is manifestly high in the acutely diabetic animals (group 1) and increases with duration of disease in the milder 'chronic' diabetics (groups 2 and 3). By 3 months glucose in the latter group has accumulated to the same extent as in the 72h 'acute' group. Fructose concentrations also tend to be higher in the acutely diabetic group, which suggests a general severe derangement of carbohydrate metabolism in this experimental model. Again, as for glucose, there is an increase in fructose with duration of diabetes (groups 2 and 3), and in all forms of diabetes concentrations are well in excess ($P<0.01$) of control values, except for liver fructose in the group-2 diabetic rats.

Glucose, fructose and sorbitol are increased up to 10-fold in nervous tissue of streptozotocin-diabetic animals, but the trends within groups by no means parallel each other. In the peripheral nerve, fructose concentrations are increased to the same extent in all forms of diabetes, whereas a 4–8-fold accumulation of sorbitol is observed with time. Both fructose and sorbitol concentrations in brain are highest in the acutely diabetic rats.

There are small but significant changes in kidney sorbitol values; they are increased over the normal in the group-1 and group-3 animals, and an increase is again observed with time, suggesting a cumulative effect. Liver sorbitol is decreased in all groups of diabetic rats, significantly in the short-term severe and long-term groups (1 and 3).

Contents of free inositol in tissues exhibit changes, either specifically associated with severe ketoacidotic diabetes or associated with progressions arising from duration of disease. Serum inositol is increased in the 72h-diabetic animals (group 1), but not in other groups, whereas a significant increase ($P<0.025$) in urinary excretion of inositol occurs in the 3-month diabetic animals (group 3). The 2-week diabetic animals (group 2) have also increased urine inositol, but this is not statistically significant.

Both brain and kidney free inositol are increased in the long-term diabetics, and liver inositol is decreased in the ketotic group (group 1) only. However, in sciatic nerve there is a progressive change. Acute short-term streptozotocin-diabetes does not evoke any change in sciatic-nerve free inositol, but there is a progressive decrease in those animals maintained for longer ($P<0.05$ for group 2 versus controls; $P<0.01$ for group 3 versus controls).

Lipid inositol

Table 3 lists the tissue concentrations of lipid-inositol found in the various groups of animals. There are only two significant differences. Sciatic-nerve lipid inositol is decreased in the group-1

Table 3. *Amounts of lipid inositol in tissue extracts of control and diabetic rats*

Lipid inositol was measured as described in the Materials and Methods section. Table 1 gives details of the groups and their treatment. Mean values are expressed as μmol/g wet wt. ±S.D. Numbers in parentheses indicate numbers of studies for each group. Student's *t* test significance limits between diabetic and control groups are given with the results; *P* values between diabetic groups are given separately to the right of the Table. *$P<0.05$; †$P<0.01$; NS, not significant ($P>0.1$).

		Diabetic			Differences between groups		
	Control	Group 1	Group 2	Group 3	1 vs. 2	1 vs. 3	2 vs. 3
Sciatic nerve	1.89±0.37 (10)	1.54±0.22 (8)*	1.88±0.39 (10)	1.86±0.29 (9)	†	†	NS
Brain	1.52±0.20 (10)	1.59±0.23 (8)	1.65±0.25 (9)	0.98±0.16 (9)†	NS	†	†
Liver	1.76±0.13 (10)	1.69±0.42 (8)	1.84±0.43 (10)	1.60±0.27 (9)	NS	NS	NS
Kidney	1.07±0.14 (5)	1.29±0.44 (8)	—	1.11±0.10 (9)	NS	NS	NS

diabetic animals, but there are no changes in the milder chronic forms (groups 2 and 3). The other change manifested is for brain lipid inositol, and in this case there is a decrease in concentration after 3 months of diabetes (group 3).

Discussion

Schein *et al.* (1971) produced a non-ketotic diabetes in rats by giving 50mg of streptozotocin/kg, but their results only cover a period of 48h after the injection. We found it necessary to lower the dose to 35mg/kg, since on the higher dose many of our rats developed ketosis and died before the 13-week study could be completed.

Glucose, fructose and sorbitol are increased 5–10-fold in nervous tissue of diabetic animals, as previously demonstrated by Gabbay *et al.* (1966) and Stewart *et al.* (1967) for alloxan-diabetic rats. This is attributed to the existence of the sorbitol dehydrogenase pathway (Gabbay, 1973) whereby excess of glucose is converted into sorbitol and then fructose by the enzymes aldose reductase and sorbitol dehydrogenase respectively. As there is limited metabolism of fructose in the nervous system via phosphorylation of fructose at C-6 (Stewart *et al.*, 1965), both fructose and sorbitol accumulate.

This accumulation of sorbitol and fructose has been associated with the development of peripheral neuropathy, as shown by decreased motor-nerve conduction velocity in animals with experimental diabetes (Gabbay, 1972; Dvornik *et al.*, 1973), although direct causality has not been established. Both decreased motor-nerve conduction velocity and inositol concentration have been noted in rat sciatic nerve 2 weeks after the induction of streptozotocin-diabetes (Greene *et al.*, 1975). Our data confirm this and show that inositol loss is progressive.

In contrast with nerve, our data show that brain free inositol increases after 3 months in the diabetic state and does not correlate with changes in fructose or sorbitol concentration as in peripheral nerve. This may reflect synthesis *de novo*, increased transport from blood to brain, decreased incorporation into lipid or enhanced phosphoinositide catabolism. It is known that transport of inositol into brain from the circulatory system occurs via a cerebrospinal-fluid pool (Spector & Lorenzo, 1975*a*) and that a slow saturable mechanism is involved. Further, inositol in brain may be derived from glucose (Hauser, 1963) and glucose 6-phosphate (Eisenberg, 1967). From their transport studies, Spector & Lorenzo (1975*b*) postulate the existence of two pools of inositol; the smaller labile pool might be affected in diabetes.

Wells *et al.* (1969) showed that galactitol, which accumulates in galactosaemia, has no effect on enzymes of inositol or phosphoinositide synthesis in preparations from rat brain. Stewart *et al.* (1969) also demonstrated the same for inositol-synthesizing enzymes in peripheral nerve. In addition, Benjamins & Agranoff (1969) have reported no effect of galactitol on brain CDP-diacylglycerol–inositol phosphatidyltransferase (EC 2.7.8.11), the enzyme that catalyses phosphatidylinositol synthesis. Thus it is likely that these enzymes are also unaffected by sorbitol, which accumulates in nervous tissue in diabetes.

A decrease in brain lipid inositol content only occurs after diabetes for 3 months (Table 3). In sciatic nerve, on the other hand, a significant decrease in lipid inositol concentration is observed only in the acutely diabetic animals (group 1). The theory has been advanced that phosphoinositides may be involved in propagation of action potentials (Kai & Hawthorne, 1969) and it has been shown that the phosphomonoester groups of both di- and tri-phosphoinositides have a high rate of turnover in nerve trunks (Yagihara *et al.*, 1969; White & Larrabee, 1973). Hence previously reported changes in motor-nerve conduction velocity may result from subtle alterations in polyphosphoinositide metabolism. The determination of total lipid inositol in the present study would not detect such changes, though Table 3 certainly suggests deranged phosphoinositide metabolism in the nervous system of the diabetic rat.

The increased renal excretion of inositol, as seen in the long-term mildly diabetic animals, has been previously documented in human diabetes (Freinkel *et al.*, 1960; Pitkanen, 1972). A decreased degradation to glucuronic acid (P. H. Whiting, unpublished work) might contribute to this, partially explaining the greater accumulation of inositol in the kidneys of the group-3 rats.

A study of inositol metabolism in the chronic 'mild' experimental diabetes described here might throw some light on diabetic neuropathy in man.

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