References and Notes

1. L. K. Georg, L. Ajello, M. A. Gordon, *Science* **114**, 387 (1951); C. A. Fuentes, F. Trespalacios, G. F. Baquero, R. Aboulafia, *Mycologia* **44**, 170 (1952); L. K. Georg, L. Ajello, C. Papageorge, *J. Lab. Clin. Med.* **44**, 422 (1954); S. A. Rosenthal and D. Furnari, *J. Invest. Derm.* **28**, 367 (1957).
2. F. Staib, *Zentralbl. Bakteriol. Abt. I Orig.* **186**, 233 (1962); *Z. Hyg.* **148**, 466 (1962); *Mycopathol. Mycol. Appl.* **19**, 143 (1963).
3. M. R. Hertz and M. Levine, *Food Res.* **7**, 438 (1942).
4. Suspend 70 grams of seed powder (pulverized in a blender) in 350 ml of distilled water. Autoclave at a pressure of 10 lb/in.^{2} (1.7 atm) for 10 minutes and filter through gauze. Procedure recommended by Dr. Margarita Silva, Columbia College of Physicians and Surgeons, New York.
5. This preliminary study was carried out under the University of North Carolina—Communicable Disease Center Laboratory Directors' Training Program, which is supported by training grant 5T1GM 567 from the National Institute of General Medical Sciences. Research is being carried out, as part of A.B.S.'s doctoral thesis requirements, at the Communicable Disease Center, Atlnata, Ga.

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Sorbitol Pathway: Presence in Nerve and Cord with Substrate Accumulation in Diabetes

Abstract. *Glucose, sorbitol, fructose, and inositol are present in peripheral nerve and spinal cord. Marked elevation of these substances occurs in these tissues in mildly diabetic animals. These alterations provide biochemical mechanisms which could be significant in the etiology of diabetic neuropathy.*

The increased conversion of glucose to fructose via the sorbitol pathway has been demonstrated in seminal vesicle and lens of animals with experimental diabetes, and in lens of diabetic patients (*1–3*). The presence of the sorbitol pathway has not previously been described for either peripheral nerve or spinal cord. Stewart *et al.* (*4*) described the presence of fructose in sciatic nerves of normal rabbits by enzymatic and paper chromatographic techniques.

This report shows the presence of sorbitol, fructose, and glucose in peripheral nerve and spinal cord, with marked elevation of these substances in mildly diabetic rats.

Rats of the Sprague-Dawley strain, weighing about 130 to 150 g, were injected intraperitoneally with alloxan in citrate buffer at a dose of 150 mg/kg of body weight. The diabetic animals used had survived 2 weeks without the use of insulin. The animals had constant access to a standard diet with liberal amounts of water and saline. This group showed a consistent 1 to 2 percent content of urinary glucose as measured by glucose oxidase test strips (Combistix, Ames). The animals in this group gained 10 to 20 g during the period of the experiment, while the average gain in the control group was 100 to 150 g. They were killed by a blow to the head followed by decapitation. Blood sugar levels at the time of death were 122 ± 14 mg/100 ml for the control group, and 440 ± 72 mg/100 ml for the diabetic group. Occasional blood sugars of some of the fasted diabetic animals were normoglycemic. The sciatic nerves were isolated and weighed within 5 to 10 minutes and spinal cord within 10 to 15 minutes of death. The tissues were kept at −60°C until used. They were homogenized in zinc sulfate, and precipitated with barium hydroxide. The protein-free filtrate was used for glucose determination by the glucose oxidase method, and sorbitol and fructose were determined by standard procedures (*2*). Part of the protein-free filtrate was evaporated to dryness, and the trimethylsilyl ethers of the sugars present were prepared by the method of Sweeley *et al.* (*5*) and examined by gas liquid chromatography on a column prepared with 15 percent ethylene glycol succinate at 160°C in an argon ionization detection system. The presence of glucose, sorbitol, fructose, and, in addition, inositol, was demonstrated.

Data obtained (Table 1) showed the presence of glucose, sorbitol, and fructose in normal sciatic nerve and spinal cord. There was a marked elevation of these substances in diabetic nerve and cord. Normal sciatic nerve was seen to contain higher levels of these substances than the spinal cord, and the effect of diabetes was more marked. There was a direct correlation, not only between the sorbitol and fructose levels, but also between the glucose and fructose levels in the tissues (correlation coefficient $r = 1$).

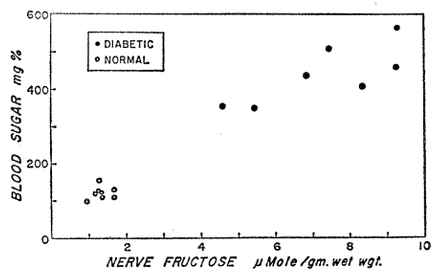

Fig. 1. Relation between blood glucose levels and nerve fructose levels.

Figure 1 shows blood glucose levels plotted against nerve fructose levels, showing a linear increase in the latter with increasing blood sugar levels. The fructose and glucose content of normal rat sciatic nerve, as found by our methods, was similar to that reported by Stewart *et al.* (*4*) for normal rabbit sciatic nerve.

A reasonable estimate of the contribution of extracellular glucose to the levels found in diabetic nerve can be made by assuming a 15 percent extracellular compartment by weight. At a mean blood sugar level of 440 mg/100 ml, the glucose present in the extracellular compartment would not exceed 4.5 μmole/g. The finding of a level of 16.97 μmole/g in diabetic nerve indicated the accumulation of a large, intracellular, free-glucose compartment. A considerable amount of inositol was also found to be present, by gas liquid chromatography, in both sciatic nerve and spinal cord.

The conversion of glucose to fructose via sorbitol has been described for lens and seminal vesicle (Fig. 2).

Kinoshita and Hayman recently demonstrated the presence of aldose reductase in spinal cord (*6*). We have obtained evidence with crude extracts of spinal cord and nerve, indicating the additional presence of polyol dehydrogenase (*7*).

The demonstration of the sorbitol pathway in sciatic nerve and spinal cord, together with the demonstration

Table 1. Content of glucose, sorbitol, and fructose in sciatic nerve and spinal cord of normal and diabetic animals.

Compound	Sugar (μmole/g wet wt. ± S.D.; $N = 12$)					
	Sciatic nerve			Spinal cord		
	Normal	Diabetic	P	Normal	Diabetic	P
Glucose	2.51 ± 1.85	16.97 ± 9.40	<0.001	0.41 ± 0.14	1.70 ± 2.31	<0.02
Sorbitol	1.88 ± 1.02	6.51 ± 1.75	<0.001	1.18 ± 0.47	1.77 ± 0.78	<0.01
Fructose	1.29 ± 0.21	6.51 ± 1.95	<0.001	0.15 ± 0.04	0.96 ± 0.23	<0.001

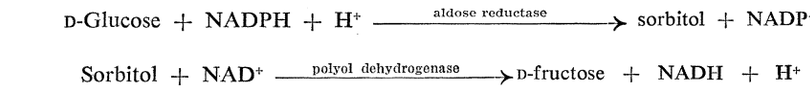

Fig. 2. Conversion of glucose to fructose via sorbitol in lens and seminal vesicle.

of marked accumulation of the products of this pathway in diabetes, has some major implications regarding the metabolism of glucose in diabetic tissues and possibly in the development of neuropathy.

Since cell membranes appear to be relatively impermeable to sorbitol and fructose, the accumulation of these substances may be due to overproduction from glucose and presumably inefficient utilization. Stewart *et al.* (*4*) claim that fructose is converted to lactate very slowly in normal sciatic nerve, and Field and Adams (*8*) showed that diabetic nerve contains no detectable glycogen. Therefore, it is probable that nerve, as opposed to liver or skeletal muscle (*9*), contains little or no specific fructokinase for initiating further glycolytic breakdown of fructose or ultimate storage as glycogen.

The additional finding of a large accumulation of intracellular glucose in diabetic nerve and cord, coupled with absent glycogen and a marked decrease in fatty acid synthesis (*10*), suggests a defect in the activation of glucose in glycolytic, oxidative, and glycogenic glucose utilization in diabetes. This would be analogous to the deficiency of specific glucokinase in diabetic liver as demonstrated by Sols *et al.* (*11*). Such a defect would then favor increased disposition of the accumulated glucose via the sorbitol pathway. Concomitantly, facilitation of glucose entry into the cell in the diabetic state is also possible. The findings of marked accumulation of glucose and the products of glucose metabolism by the sorbitol pathway is in keeping with this view.

Another possible consequence of the accumulation of sorbitol and fructose and their inability to escape from the intracellular compartment may be a harmful osmotic effect, for Kinoshita (*2*) showed that in galactosemic animals a similar process leading to accumulation of dulcitol and fructose caused water accumulation and Na^+ and K^+ alterations in the early cataractous lens. Greater amounts of cell energy are used for maintenance of osmotic equilibrium and Na^+–K^+ content. Whether a similar mechanism plays a role in some forms of diabetic neuropathy has not yet been determined.

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References and Notes

1. T. Mann and U. Parsons, *Biochem. J.* **46**, 440 (1950).
2. J. H. Kinoshita, L. O. Merola, E. Dikmak, *Exptl. Eye Res.* **1**, 405 (1962).
3. A. Pirie and R. Van Heyningen, *ibid.* **3**, 124 (1964).
4. M. A. Stewart and J. V. Passonneau, *Biochem. Biophys. Res. Comm.* **17**, 536 (1964).
5. C. C. Sweeley, R. Bentley, M. Makita, W. W. Wells, *J. Am. Chem. Soc.* **85**, 2497 (1963).
6. J. H. Kinoshita and S. Hayman, in preparation.
7. In preparation.
8. R. A. Field and L. C. Adams, *Medicine* **43**, 275 (1964).
9. S. Hollman, *Non-Glycolytic Pathways of Metabolism of Glucose* (Academic Press, New York, 1964), pp. 28–29.
10. R. A. Field and L. C. Adams, *Biochim. Biophys. Acta*, in press.
11. E. Vinuela, M. Salas, A. Sols, *J. Biol. Chem.* **238**, PC1175 (1963).
12. We are grateful to Dr. J. H. Kinoshita for his interest and encouragement in this project. Supported in part by PHS grants NB-04063-04 and TI AM 5072.

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Plasma Membranes: Phospholipid and Sterol Content

Abstract. *The molar ratios of sterol to phospholipid in plasma membranes of five different types of rat cells range from 0.24 to 1.32. The composition of the plasma membrane of a cell has no fixed relation to that of the mitochondria. Thus the structure of cellular membranes shows both tissue and functional specificity.*

Fundamental questions raised as a result of electron microscopic studies of tissues are those of the origin of cell membranes and of the relation between structures within a single cell and between similar structures in different types of cell. Robertson (*1*) proposed that all cell membranes have a common basic structure and possibly a common origin, whereas Sjöstrand (*2*) has emphasized morphological differences. Many workers have found that nerve myelin and erythrocyte membranes have a relatively high sterol content, and more recently a similar finding was reported for intestinal microvilli (*3*). In that these all consist essentially of cell plasma membranes, we have examined a number of such membrane preparations from one species for sterol content. The sterol content is of interest because myelin is often taken as a general model for plasma membranes. We have also made comparisons with mitochondria and with liver microsomal membranes.

The following preparations were all obtained from the rat: erythrocyte ghosts (*4*), brain myelin (*5*), intestinal microvilli (*6*), muscle-cell membranes (*7*), and liver-cell plasma membranes (*8*). Mitochondria from liver, intestinal mucosa, muscle, and brain were also examined; the brain mitochondria were purified (*5*) to free them from myelin. In addition, we applied the method of Wallach and Ullrey (*9*), for obtaining Ehrlich ascites cell plasma membranes, to liver. As with the ascites cells, three suspended subfractions (numbered in order of increasing density) as well as a sediment were obtained from the crude microsomal pellet.

To ensure complete lipid extraction in the presence of sucrose, the method of Hanson and Olley (*10*) was used. Individual classes of lipids were separated by silicic acid chromatography (*11*) followed by determination of sterol (*12*) and phospholipid (*13*). Protein was estimated from the nitrogen content (micro-Kjeldahl), and the values were checked in several cases by use of the Folin-Ciocalteu reagent.

From the results (Table 1) only one finding is common to all membrane preparations—the small percentage of sterol present in the esterified form. The proportion of protein to phospholipid varies ninefold among the different plasma membranes. The largest proportion of protein was found in the intestinal microvilli, which are presumably the most active of the membranes and need more specialized proteins such as enzymes and carriers, and the smallest proportion of protein was found in myelin which presumably has lost all of its associated specialized proteins and consists only of the bare