IDENTIFICATION OF SORBITOL IN MAMMALIAN NERVE*

William R. Sherman and Mark A. Stewart

Department of Psychiatry
Washington University School of Medicine
St. Louis, Missouri

Received January 24, 1966

Hers (1960) has shown that fructose in the seminal vesicles is formed from glucose via sorbitol. Van Heyningen (1959), Kuck (1961) and Kinoshita et al. (1963) have reported evidence that the same pathway is present in the lens. The finding of relatively high levels of free fructose in mammalian nerve (Stewart and Passonneau, 1964) raised the question whether the "sorbitol pathway" also operates in nerve. In this paper we report the identification and measurement of sorbitol in mammalian nerve.

Materials. Sciatic nerves were taken from 200 g Holtzman rats, 2.5 kg albino rabbits, and adult macaque monkeys, with the animal under nembutal anesthesia. The nerves were frozen at once in liquid N_2 and later cleaned and weighed in a room at -15°. For enzymatic assays $HClO_4$ extracts of the nerves were prepared in the way described by Lowry et al. (1964). For gas chromatography the samples were extracted in water as follows. 200 µl of water at 2° was added to the frozen sample (25 to 50 mg), which was immediately heated at 100° for three minutes. The sample was then homogenized, deproteinized with $ZnSO_4$ according to the method of Somogyi (1939), and then lyophilized.

Assay Methods.

(a) Gas Chromatography. Carbohydrates in the lyophilized extracts were converted to their trimethylsilyl ethers by the technique described by Sweeley,

*This work was supported by the following USPHS grants: GM-21,863, NB-05159; MH-13,340, MH-08765, NB-05463.

Bentley, Makita and Wells (1963), adding about 50 µl of the reagent to each tube. The tubes were capped and allowed to stand at room temperature for 48 hours. The reaction mixtures were then centrifuged at low speed and 2 µl aliquots of the supernatant injected into the gas chromatograph with a 10 µl Hamilton syringe fitted with a Chaney adapter. Chromatography was carried out on either 15% ethylene glycol succinate (EGS, on 80-100 mesh Gas-Chrom P from Applied Science Laboratories, State College, Pa. in a 12' X 0.25" glass tube at 160°) or on 3% silicone rubber (SE-52 on the same support in a 6' X 0.25" steel tube at 170°). Helium carrier (100 ml/minute) and flame ionization detector were used on an F and M model 810 gas chromatograph.

Quantitation of the gas chromatographic data was by peak height, the sugar measured being compared with its counterpart in a mixture of standard sugars run on the same instrument under identical conditions. Good separation was obtained for all sugars measured. Linear correlation between peak height and weight of sugar derivatives was demonstrated over the range 5 µg to 0.005 µg for fructose, glucose, sorbitol and myo-inositol. In five experiments where measured quantities of sorbitol were added to samples of nerve and serum, recoveries were 100% (S.D. 6%).

(b) Enzymatic Assay. Sorbitol was measured by a fluorimetric modification of the method described by King and Mann (1959). The reagent consisted of 0.4 mM DPN and 2 mM $MgCl_2$, in 50 mM glycine buffer at pH 9.6. Sorbitol, from standard solution or from extracts, was added to the reagent to give a concentration of about 1.5 µM. Sorbitol dehydrogenase (Boehringer and Sons) was added, and the appearance of DPNH followed fluorimetrically. Glycerol was previously removed from the enzyme suspension by dialysis against a phosphate buffer because it gave a high blank reading. Under these conditions the assay was linear for sorbitol concentrations in the final reagent from .5 µM to 3 µM in the presence of fructose at concentrations twenty times greater. The sorbitol dehydrogenase also catalyzes the oxidation of xylitol and ribitol but does not react with galactitol, mannitol, arabitol or eryth-

ritol. The first order rate constants for the reactions with xylitol, sorbitol, and ribitol were .41, .17 and .046 min^{-1} respectively.

Results. Gas Chromatography of nerve extracts on EGS, showed a substance with the same elution time relative to glucose as sorbitol (Rg=1.33±.02). Chromatography on a non-polar column (SE-52) also demonstrated the presence of a substance of the same retention time as sorbitol (Rg=1.24±.02). In the enzymatic assay system, extracts of nerve gave a time curve for DPNH appearance which was identical with that of standard sorbitol. The levels of sorbitol measured by the two methods are given in Table I.

Table I

Sorbitol Levels in Sciatic Nerve
(mmoles/kg. wet wt.)

	Enzymatic			Gas Chromatography		
	N	Mean	S.D.	N	Mean	S.D.
Rat	33	.18	.026	3	.18	-
Rabbit	8	.11	.018	7	.17	.02
Monkey	2	.15	-	2	.18	-

To confirm the chromatographic identification of sorbitol, nerve samples were extracted in 50 mM Na_2CO_3 Buffer (pH 9.5), and aliquots incubated with and without sorbitol dehydrogenase, in the presence of 5 mM DPN^+ and 2 mM $MgCl_2$, before being prepared for gas chromatography. Table II shows that in monkey and rat sorbitol was almost eliminated, while in rabbit a residue eluting at Rg 1.33 remained. It is possible either that the substance remaining at Rg 1.33 is a residue of sorbitol, unoxidized due to a peculiarity of rabbit tissue, or that it is some other material eluting at the same time. In support of the latter view is the fact that if the mean gas chromatographic value for rabbit nerve sorbitol presented in Table I is

reduced by 40% (the average residual portion eluting at Rg 1.33) it becomes 0.11 mmoles/kg wet, the amount measured enzymatically. Due presumably to sorbitol dehydrogenase activity remaining in the tissue, the sorbitol levels of all the control tissues were depleted below the usual levels found in tissues of the same species.

Table II

Depletion of Sorbitol in Sciatic Nerve by Sorbitol Dehydrogenase (mmoles/kg. wet wt.)

	N	Control	Treated	% Depletion
Rat	2	.090	<.006	>90
Rabbit	7	.10 (.063-.214)	.039 (.018-.064)	60 (79-36)
Monkey	2	.074	<.01	>85

What appears to be sorbitol was also measured in brain and serum by gas chromatography, using the same methods for tissue preparation as for nerve. In rat brain the level of sorbitol was 26 µmoles/kg wet wt (N=5, S.D.=4). In rat serum the levels were 5 µmoles/liter or less (N=5), barely detectable at the maximum sensitivity of the method.

Myo-inositol was also found in all the tissues examined. In rat brain the levels were 5.44 mmoles/kg wet wt (N=5, S.D.=0.42) in good agreement with the values reported by Wells, Pittman and Wells (1965) who obtained 5.96 (N=10, S.D.=0.19). Serum levels in rat were 90 µmoles/liter (N=5, S.D.=22). In rabbit sciatic nerve myo-inositol was present in a concentration of 5.51 mmoles/kg wet (N=7, S.D.=0.23).

Discussion. The finding that sorbitol is present in nervous tissue at levels considerably greater than in serum is evidence that a glucose to sorbitol to fructose pathway operates in the nervous system as in the lens and seminal vesicle. In addition we have measured enzymatic activity in rat nerve catalyzing the reactions:

$$\text{Glucose} + \text{TPNH} + H^+ \rightleftharpoons \text{Sorbitol} + TPN^+$$

$$\text{Sorbitol} + DPN^+ \rightleftharpoons \text{Fructose} + \text{DPNH} + H^+$$

With glucose as substrate (100 mM PO_4 buffer, pH 7.1, 27°) the aldose reductase activity is 0.8 mM/Kg wet wt/hr. With sorbitol as substrate(100 mM Na_2CO_3 buffer, pH 9.6, 27°) the sorbitol dehydrogenase activity is 0.5 mM/Kg wet wt/hr. The K_m under these conditions for sorbitol is .14 mM, and for glucose about 9 mM.

We have reported evidence that fructose in nerve is confined to the Schwann cell compartment (Stewart, Passonneau and Lowry, 1965). It seems likely that the sorbitol pathway is a characteristic of Schwann cell, rather than axonal metabolism. However fructose and sorbitol are also present in brain, though at much lower concentrations, showing that this pathway may operate in other types of cells in the nervous system.

While this paper was in preparation the work of Gabbay et al. (1966) was published describing the measurement of sorbitol in sciatic nerve and spinal cord. Our findings differ from theirs in that we find one tenth the sorbitol in sciatic nerve that these authors report. This difference is perhaps explicable on the basis that their method, a periodate oxidation procedure, is not specific for sorbitol. In our determination, two methods, each highly specific for sorbitol, have been found to be in substantial agreement.

It is a pleasure to acknowledge the technical assistance of T.H. Hougen and M.M. Kurien in this work.

REFERENCES

Gabbay, K.H., Merola, L.O. and Field, R.A., Science, 151, 211 (1966).
Hers, H.G., Biochim. Biophys. Acta, 37, 127 (1960).
King, T.E. and Mann, T., Proc. Roy. Soc. B., 151, 226 (1959).
Kinoshita, J.H., Futterman, S., Satoh, K., and Merola, L.O., Biochim. Biophys. Acta, 74, 340 (1963).
Kuck, J., Arch. Ophthal. 65, 100, (1961).

Lowry, O.H., Passonneau, J.V., Hasselberger, F.X. and Schultz, D.W., J. Biol. Chem., 239, 18 (1964).
Pirie, A. and Van Heyningen, R., Exp. Eye Res., 3, 124 (1964).
Stewart, M.A. and Passonneau, J.V., Biochem. Biophys. Res. Comm., 17, 536 (1964).
Stewart, M.A., Passonneau, J.V. and Lowry, O.H., J. Neurochem., 12, 719 (1965).
Somogyi, M., J. Biol. Chem., 86, 655 (1939).
Sweeley, C.C., Bentley, R., Makita, M., and Wells, W.W., J. Amer. Chem. Soc., 85, 2497, (1963).
Van Heyningen, R., Nature, 184, 194 (1959).
Wells, W.W., Pittman, T.A., and Wells, H.J., Anal. Biochem., 10, 450 (1965).