FREE SUGARS IN ALLOXAN DIABETIC RAT NERVE*

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We have previously identified fructose and sorbitol in mammalian peripheral nerve (Stewart and Passonneau, 1964, and Sherman and Stewart, preceding paper). These free sugars are also present at substantial levels in the normal lens, and at greatly increased levels in the lenses of diabetic patients (Pirie and Van Heyningen, 1964) and of experimental diabetic animals (Van Heyningen, 1959). This accumulation of sorbitol and fructose in the lens is associated with cataract formation.

We have been interested in a possible relationship between fructose and sorbitol levels in nerve, and the lesions that occur in the nerves of diabetic patients, and have measured changes in the levels of these sugars in nerves of diabetic animals.

Method. 200 g female Holtzman rats were fasted 48 hours, then given alloxan (120-150 µg/Kg) intraperitoneally. The animals were tested for glycosuria 48 hours later with a glucose oxidase impregnated paper indicator, and those showing 2% or more glucose in the urine were diagnosed as diabetic; these animals received no further treatment. Control groups were made up of (1) rats that had no treatment, and (2) rats that were given the same dose of alloxan as the test group, but had not been fasted. The latter animals did not become diabetic.

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6 days, 2 weeks, 5 weeks, and 6 weeks after the injection of alloxan, the sciatic nerves were removed from 6 to 8 diabetic rats, under nembutal anesthesia. Nerves were taken from the alloxan treated controls 6 days after the alloxan injection. The nerves were frozen at once in liquid N_2; samples were later cleaned of blood and connective tissue, and weighed in a room at -15°. The samples were extracted in 3M $HClO_4$ at -10° as described by Lowry et al. (1964). Glucose and fructose in the extracts, and glucose in the sera, were measured enzymatically (Lowry et al., 1964, and Stewart and Passonneau, 1964). Sorbitol in the extracts was measured enzymatically (Sherman and Stewart, preceding paper); sera were analyzed for sorbitol by gas chromatography.

Results. The results are given in the table.

	N	Sciatic Nerve (mmoles/Kg wet wt.)			Serum (mmoles/liter)
		Glucose	Fructose	Sorbitol	Glucose
Normal Controls	8	2.78± .09	1.11± .05	0.18± .01	9.21± .34
Alloxan Controls	8	3.36± .04	1.22± .09	0.19± .01	10.21± .27
6 days diabetic	8	16.2 ± .7	7.16± .38	3.92± .26	40.1 ±1.9
2 weeks diabetic	8	16.5 ± .9	6.79± .47	3.09± .29	40.5 ±2.0
5 weeks diabetic	6	17.3 ±1.9	8.69± .50	3.49± .26	39.5 ±5.3
6 weeks diabetic	6	16.9 ±1.8	7.86±1.2	3.66± .63	39.2 ±2.6

Sorbitol was present in both normal and diabetic sera at a very low level (less than 5 μM/L).

Discussion. The results show marked increases in the levels of all three sugars in the nerves of diabetic animals. The increases have taken place

by the sixth day after the injection of alloxan, and the levels then remain constant through the time of the experiment. Glucose and fructose in the diabetic rat nerves increased about seven-fold, but sorbitol levels increased almost twenty-fold. If the substrates in the glucose to fructose pathway are in equilibrium in tissue, the relatively greater increase in sorbitol level may imply (a) a change in ratio of oxidized to reduced pyridine nucleotides in the diabetic nerve, (b) a drop in intracellular pH, (c) that part of the increased sorbitol content is in a "compartment" separated from the enzymes, or (d) a combination of these effects.

The diabetic animals had all lost weight, and showed markedly increased food and water intake, and urine output. None, however, gave any evidence of peripheral nerve lesions. There is therefore no reason as yet to relate the increased sugar concentrations found in diabetic rat nerve to the neuropathy of diabetic patients. However Eliasson (1964) has found decreased conduction velocity in the nerves of alloxan diabetic rats, showing that there is a physiological disturbance in such animals.

An important technical point is that the substrate levels in diabetic nerves were found to change rapidly unless the nerves were kept frozen before extraction. Four diabetic nerves were divided; one half extracted as described above, and one half extracted in .3M $HClO_4$ at 2°. Fructose levels were 25% higher in the samples extracted at 2°. We have also found that samples of diabetic nerve extracted in water at room temperature for 15 min. showed complete disappearance of glucose and sorbitol and a corresponding increase in fructose.

While we were preparing this paper Gabbay *et al.* (1966) published a report of increased sorbitol levels in alloxan diabetic rat nerve. Our data differ from those presented by these investigators chiefly in the measurements of sorbitol; we found the level of sorbitol to be one tenth that reported by Gabbay *et al.* in normal nerve, and one half that found in diabetic nerve. A possible reason for this discrepancy is discussed in the preceding paper.

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