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Increase of Urinary and Tissue Hexosamine in Streptozotocin Diabetic Rats (37798)

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(Introduced by Piero P. Foà)

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The PAS-positive materials that accumulate in diabetic microangiopathy are believed to represent a collagen-like glycoprotein in the renal glomerulus (1) and a mucopolysaccharide in the arterioles (2). Hexosamine has aroused particular interest because it is an important component of both glycoproteins and mucopolysaccharides.

Spiro has reported that in spite of the great impairment of glycogen synthesis in diabetes, glucosamine synthesis was not decreased (3). Indeed, an increase in the hexosamine content of various tissues and serum of diabetic animals and patients has been reported (4–6); however, these observations have not been sufficient to substantiate the claim that glucosamine synthesis is elevated in diabetes mellitus. We wish to report a significant increase in urinary bound hexosamine in experimental diabetes.

Materials and Methods. Male Sprague–Dawley rats, weighing between 150 and 170 g were maintained in individual metabolic cages on Oriental laboratory chow and water ad lib. After at least 4 days they were fasted overnight and were given a single injection of streptozotocin (Upjohn Co., Kalamazoo, Mich.) into the tail vein, under light ether anesthesia. Streptozotocin was dissolved in normal saline immediately prior to use. The pH was adjusted to 4.5 with 0.1 *M* citrate buffer and the dose was 65 mg/kg. Two months after the injection of streptozotocin the diabetic rats (mean fasting serum glucose greater than 250 mg%), were fasted overnight and sacrificed by drawing blood from the iliac vein under pentobarbital anesthesia.

The 24-hr. urine was collected in bottles containing 0.1% sodium azide. The urinary glucose was measured by a modified method of Benedict (7). The determination of urinary hexosamine was carried out at 4° as follows: after centrifugation at 1000*g* for 10 min and again at 10,000*g* for 20 min, the supernatant was concentrated to roughly 0.5 ml in a cellophane tube imbedded on polyvinyl pyrrolidone powder and dialyzed extensively overnight against 2000 ml of 0.005 *M* phosphate buffer, pH 7.0 with several changes. The volume of the dialyzed solution was adjusted to 2 ml with distilled water, 2 ml of 4 *N* HCl were added and the solution was hydrolyzed at 100° for 16 hr. After centrifugation at 10,000*g* for 20 min, the supernatant was dried in vacuum over pellets of phosphorus pentoxide

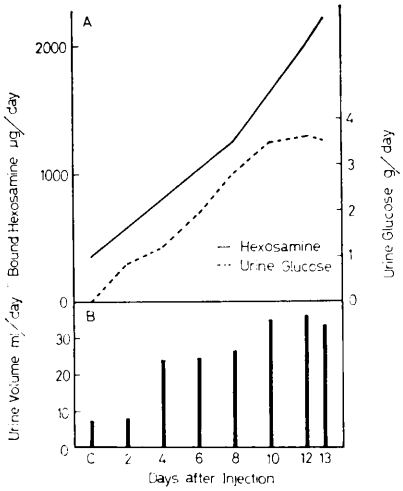

FIG. 1. Urinary excretion of bound hexosamine and glucose (A) and urine volume (B) in rats after the injection of streptozotocin (65 mg/kg body wt). Average of two typical animals.

TABLE I. Ratio of Glucosamine to Galactosamine of Tissue Hexosamine and Urinary Bound Hexosamine.

	Liver	Kidney	Urine
Normal	15.0 ± 1.0	15.8 ± 1.1	16.8 ± 1.2
Diabetic	13.3 ± 0.8	14.9 ± 0.9	14.5 ± 0.9

Means ± SE are calculated from values for three rats.

and sodium hydroxide. Two milliliters of distilled water were used to dissolved the dried material. This solution was applied to a Dowex 50 W × 8 column (1 × 5 cm), previously washed with distilled water. After washing with 15 ml of distilled water, the hexosamine fraction was eluted with 5 ml of 2 *N* HCl and neutralized with 4 *N* NaOH. Hexosamine was measured according to Boas (8), using a glucosamine standard. This modified Elson-Morgan reaction could be used to determine urine and tissue hexosamines since substances such as neutral sugars, which give false positive results, have been excluded by column chromatography.

The total hexosamine content of serum and tissues was measured as described above using 0.2 ml of serum mixed in 4 ml of 2 *N* HCl and 500 mg of liver or kidney tissue homogenized in 5 ml of 2 *N* HCl. Dowex 50 W × 8 chromatography (1 × 24 cm) equilibrated with 0.3 *N* hydrochloric acid was used for the separation of glucosamine and galactosamine (9) to calculate the ratio of glucosamine to galactosamine. Hepatic glutamine-fructose 6-phosphate-aminotransferase (glucosamine synthetase, EC 2.6.1.16) was assayed as described by Ghosh *et al.* (10), using a Hitachi spectrophotometer model 124 at 25°.

Results. Figure 1 shows that during the first 10–13 days after the injection of streptozotocin, there was an increase in urinary bound hexosamine, glucose and volume. After this point, urinary bound hexosamine remained elevated throughout the remaining 2 mo of study.

The ratio of glucosamine to galactosamine in liver, kidney and urine was greater than 10 in both diabetic and control rats (Table I).

The hexosamine content of the liver and urine but not that of the kidney and serum was significantly higher in the diabetic rats than in their age-matched controls (Table II). The activity of glucosamine synthetase was not elevated in the tissue of the diabetic rats (Table III).

Discussion. Glucosamine is readily incorporated into macromolecules (11) and almost all tissue hexosamines exist in the bound form (12). Glucosamine incorporated into seromucoid has been shown to be elevated in the diabetic state (13); these facts and our data suggest that the increase in hexosamine might be due not only to an increase in hexosamine biosynthesis, but also to an enhanced biosynthesis of glucosamine-containing macromolecules. This in turn, may be related to the accumulation of PAS positive material in the microangiopathic lesions of diabetes. While the degradation rate of radioactive glucosamine in the liver of diabetic rats does not appear abnormal (3), the possibility of decreased degradation of bound glucosamine has not been excluded by this study.

Summary. Male Sprague–Dawley rats were made diabetic with streptozotocin (65 mg/kg, iv). Severe polyuria and glycosuria were accompanied by a marked increase in the 24-hr urinary excretion of bound hexosamine. There was a slight but significant increase of liver hexosamine in the treated rats compared with their age matched controls, although the ac-

TABLE II. Tissue Hexosamine Content and Urinary Bound Glucosamine Excretion of Normal and Diabetic Rats.

	Hexosamine content			Urinary excretion of bound hexosamine (μg/day)
	Serum (μg/100 ml)	Liver (μg/g tissue)	Kidney (μg/g tissue)	
Normal	115 ± 6(14)	831 ± 36(6)	1410 ± 128(6)	499 ± 68(11)
Diabetic	111 ± 5(8)	1120 ± 60(5)	1650 ± 67(6)	1460 ± 170(14)

Mean ± SE N: Normal D: Diabetic

TABLE III. Liver Glucosamine Synthetase Activity of Normal and Diabetic Rats.

	μmoles Glucosamine formed/hr/g tissue	μmoles Glucosamine formed/hr/100 g body wt
Normal	3.98 ± 0.22(6)	12.3 ± 1.1(6)
Diabetic	3.70 ± 0.24(8)	14.5 ± 1.4(8)

Mean ± SE.
Numbers of animals in parentheses.

tivity of hepatic glucosamine synthetase was unchanged. The results support the hypothesis that glucosamine synthesis and incorporation into macromolecules are enhanced in this type of experimental diabetes.

1. Beisswenger, P. G., and Spiro, R. G., Science **168**, 596 (1970).
2. Cohen, M. P., and Foglia, V. G., Diabetes **19**, 639 (1970).
3. Spiro, R. G., Ann. N.Y. Acad. Sci. **82**, 366 (1959).
4. Walker, F., and Patrick, R. S., Diabetes **17**, 105 (1968).
5. Patrick, R. S., and Walker, F., Diabetologia **5**, 29 (1969).
6. Alderberg, D., Wang, C-I., Rifkin, H., Berkman, J., Ross, G., and Weinstein, C., Diabetes **5**, 116 (1956).
7. Benedict, S. R., J. Amer. Med. Ass. **57**, 1193 (1911).
8. Boas, N. F., J. Biol. Chem. **204**, 553 (1953).
9. Gardell, S., Acta Chem. Scand. **7**, 207 (1953).
10. Ghosh, S., Blumenthal, H. J., Davidson, E., and Roseman, S., J. Biol. Chem. **235**, 1265 (1960).
11. Kohn, P., Winzler, R. J., and Hoffmann, R. C., J. Biol. Chem. **237**, 304 (1962).
12. Kent, P. W., and Whitehouse, M. W., "Biochemistry of Aminosugars." Butterworths Scientific Publication, London (1955).
13. Capreol, S. V., Sutherland, L. E., and Hanimyan, D. A., J. Endocrinol. **50**, 355 (1971).

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