Sulfate^{35} Uptake and Incorporation into Aortic Mucopolysaccharides in Experimental Diabetes (34218)

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Several investigators have reported that sulfate incorporation into acid mucopolysaccharides is depressed in diabetic animals. Using autoradiographical techniques, Kranz *et al.* (1) demonstrated less shadowing in corresponding mucopolysaccaride-staining areas of aortas from diabetic rats as compared with controls after intraperitoneal ^{35}S. Photometric measurements of autoradiograms revealed an inhibition of radiosulfate incorporation into the acid mucopolysaccharides of aortas of alloxan diabetic rats which could be partially cancelled by insulin (2). Schiller and Dorfman found that the uptake of labeled sulfate by the chondroitin sulfate fraction in the skin of alloxan diabetic animals was reduced and insulin treatment restored the values to normal (3). Ichida and Kalant reported that the incorporation of ^{35}S into aortic sulfated glycosaminoglycans 24 hr after intravenous sulfate^{35} was consistently lower in alloxan diabetic than in normal rats (4).

However, it is not known whether this depressed sulfate uptake is the result of decreased synthesis, increased degradation, or both. As part of a study concerning the metabolism of aortic wall acid mucopolysaccharides in experimental diabetes, the uptake and incorporation of radiosulfate in the mucopolysaccharide fraction of this tissue was therefore determined.

Materials and Methods. A 95% pancreatectomy was performed according to the technique of Foglia (5) on male white rats (Institute strain) weighing 80–120 g, and development of diabetes was followed by blood sugar levels after 7-hr fasting. Diabetic animals (blood sugar 150–200 mg/100 ml) were sacrificed 5-months postpancreatectomy, when diabetes was well established, and were matched with control animals of the same age and sex. Animals were sacrificed 2, 4, 6, 24, 48, 72 hr, and 4 and 7 days after an intraperitoneal injection of 100 μCi of (^{35}S) Na_2SO_4. The aortas were excised, freed of adventitial fat, incised longitudinally and cut transversely into 0.5-cm segments. The tissue was washed in 3 successive 50-ml portions of 0.026 *M* Na_2SO_4, defatted with acetone, and dried with ether. Isolation of the mucopolysaccharides was performed according to the method of Schiller *et al.* (6), by proteolytic digestion and extensive dialysis. Following lyophilization, the samples were reconstituted in 0.4 ml of *n*-propanol:1% cetylpyridinium chloride (2:1), from this 0.04 ml was removed for determination of uronic acid (7) and 0.04 ml was pipetted directly into 10 ml of Bray's solution (8) and the radioactivity was determined in a liquid scintillation counter. The remainder of the samples was subsequently separated on cellulose columns, and these results will be the subject of a separate report.

Results. There was a marked increase in the early uptake of ^{35}S in the aortas of diabetic animals, which reached a peak at 4 hr and rapidly declined by 6 hr to normal levels (Fig. 1). In order to eliminate the possibility that increased absorption from the intraperitoneal route in diabetic animals was responsible for this finding, blood specimens were obtained every 15 min for 1 hr and the radioactivity was determined in aliquots of the serum. Peak activity was seen at 30 min in both normals and diabetics, and there was no difference in the rate of absorption, proportional to relative body weight, between

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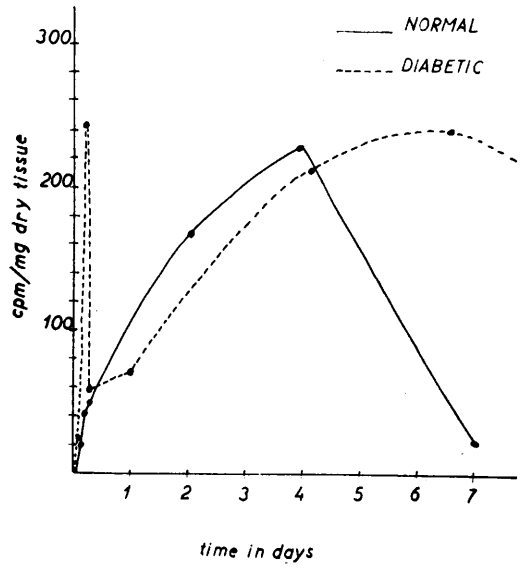

FIG. 1. Sulfate[35] uptake in aortic mucopolysaccharides in normal and diabetic rats.

the two groups of animals. After 6 hrs, sulfate uptake curve of the diabetic rats paralleled that of the normals, except that it reached its peak several days later (Fig. 1).

Sulfate incorporation (cpm/μg of uronic acid) was also greatly increased at 4 hr, rapidly declining to below normal levels at 24 hr. After 24 hr, the slope of the incorporation curve in the diabetics was similar to that of the controls, except that values were lower for the synthetic phase and higher for the degradative phase (Fig. 2).

Discussion. The striking 4-hr peak in aortic sulfate[35] uptake seen in diabetes was an unexpected finding. The rapid fall in specific activity to below normal levels by 24 hr indicates a compartment of intense metabolic turnover in the mucopolysaccharide fraction which is unmasked in the absence of insulin. Since these animals were not markedly hyperglycemic, growth hormone was probably not greatly depressed, and an early effect of the growth hormone-dependent serum sulfation factor could account for these findings (9). Although insulin *in vitro* causes significant stimulation of sulfate uptake by costal cartilage from hypophysectomized rats, it has no effect on uptake in tissue from growth hormone-treated hypophysectomized rats (9, 10). In the normal animal, insulin and sulfation factor may thus act competitively to stimulate the same site, and in the pancreatectomized animal a greater efficiency of the latter in promoting sulfation would become evident. Further experiments to test this hypothesis are currently in progress.

These results are particularly interesting in view of the high incidence of atherosclerotic cardiovascular disease in diabetes. It has been shown that sulfate uptake after 4-hr incubations is increased in atherosclerotic vessels (11), and that there is an increased ^{35}S uptake and increased turnover of glycosaminoglycans in atherosclerosic areas of aortas from animals fed high-fat diets (4).

Following the early peak, the synthesis of sulfated compounds is decreased in diabetes. This finding was substantiated by the prolonged uptake curve, indicating poor utilization of available sulfate. The slow degradation phase seen in diabetes suggests a decreased turnover of sulfated mucopolysaccharides as well.

Summary. Sulfate[35] uptake and incorporation was markedly increased in diabetic rats 4 hr after an intraperitoneal injection of labeled sulfate. Following this early peak, the synthesis of sulfated compounds was decreased in experimental diabetes.

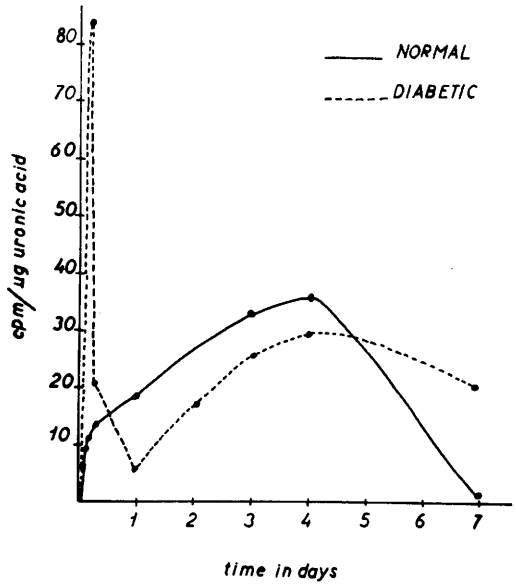

FIG. 2. Sulfate[35] incorporation into aortic mucopolysaccharides in normal and diabetic rats.

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1. Kranz, D., Kunz, J., and Keim, J., Z. Ges. Inn. Med. **23**, 40 (1968).

2. Braselman, H., Kunz, J., and Keim, O., Acta Biol. Med. Ger. **17**, 232 (1966).

3. Schiller, S. and Dorfman, A., J. Biol. Chem. **227**, 625 (1957).

4. Ichida, T. and Kalant, N., Can. J. Biochem. **46**, 249 (1968).

5. Foglia, V. G., Rev. Soc. Arg. Biol. **20**, 21 (1944).

6. Schiller, S., Slover, G. A., and Dorfman, A., J. Biol. Chem. **236**, 983 (1961).

7. Dische, Z., *in* "Methods in Carbohydrate Chemistry," Vol. 1, p. 497. Academic Press, New York (1962).

8. Bray, G. A., Anal. Biochem. **7**, 279 (1966).

9. Salmon, W. D., Jr. and Daughaday, W. H., J. Lab. Clin. Med. **49**, 825 (1957).

10. Salmon, W. D., Jr., J. Lab. Clin. Med. **56**, 673 (1960).

11. Curran, R. C. and Crane, W. A. J., J. Pathol. Bacteriol. **84**, 405 (1962).

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