Effect of Insulin *in Vitro* on ^{14}C-Acetate and ^{35}S-Sulfate Uptake into Aortic Mucopolysaccharides in Normal and Diabetic Rats[1] (35000)

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The effect of insulin on the metabolism of mucopolysaccharides remains unclear. Schiller and Dorfman found that the uptake of ^{14}C-acetate or glucose by hyaluronic acid and chondroitin sulfate, as well as the uptake of ^{35}S-sulfate by chondroitin sulfate in the skin of alloxan diabetic rats was about one-third that of normals, while the effect of insulin in normal animals was variable and not too striking (1). In contrast, Spiro found that the livers of alloxan diabetic rats were able to synthesize the glucosamine component of liver and serum glycoproteins at a normal rate, and concluded that the biosynthesis of glucosamine from glucose is not under the metabolic regulation of insulin. Furthermore, he postulated that in insulin deficiency, glucose is shunted from insulin-dependent pathways to those that don't require insulin, and, therefore, diabetes results in normal or even increased glycoprotein synthesis (2).

Although it is generally agreed that sulfate incorporation into mucopolysaccharides is depressed in chronic diabetes (3–7), the results of *in vitro* studies concerning the effect of insulin on sulfation are conflicting. Salmon and Daughaday reported that insulin *in vitro* causes significant stimulation of sulfate uptake by costal cartilage from hypophysectomized rats and from normal fasted rats, although it has no effect on uptake in tissues from growth hormone-treated hypophysectomized rats (8, 9). Wettenhall *et al.* observed only marginal effects of insulin on ^{35}S-sulfate incorporation into growing bones in a steady-state culture system (10). These results suggest that insulin, like growth hormone (11), might be effective in enhancing sulfation only *in vivo*, and that insulin *in vitro* is without effect on normal tissue.

The present investigation was undertaken to determine the effects of insulin on ^{14}C-acetate and ^{35}S-sulfate uptake *in vitro* into mucopolysaccharides in normal and diabetic tissue. Rat aortas were used for these studies since we had previously found that sulfation in this tissue is quite sensitive to changes in the circulating insulin (3, 12).

Materials and Methods. Male white rats (Institute strain) were used for all studies. A 95% pancreatectomy was performed on animals weighing 80–120 g, and the development of diabetes was followed by blood sugar levels after 7 hr of fasting. Operated animals were sacrificed 5–6 months postpancreatectomy, when diabetes was well established, and were matched with control animals of the same age and sex.

Aortas were excised immediately after sacrifice and were placed in cold physiologic saline during preparation. The tissue was freed of adventitial fat, incised longitudinally, and cut transversely into 0.5-cm segments. Aortas from normal or diabetic rats were respectively pooled, and portions of tissue were then distributed to previously prepared incubation beakers. The amount of tissue used for the individual incubations was equivalent to about one half of a single rat aorta (5–15 mg dry weight).

The basal medium was a solution of Krebs phosphate buffer, pH 7.4, containing 200 mg/100 ml glucose. Incubations were performed in 5 ml of this medium, to which 1000 units of penicillin, 2 mg of streptomycin, and 10 μCi of either (^{35}S) Na_2SO_4 or ^{14}C-acetate had been added. The concentration of insulin employed was 20 μg/ml (0.54 units/ml). All incubations were carried out for 24 hr at 37° using air as the gas phase.

[1] Aided by Grant 3493/68 from the Consejo Nacional de Investigaciones Cientificas Técnicas.

TABLE I. ^{14}C-Acetate Uptake *in Vitro* into Rat Aortic Mucopolysaccharides.[a]

Group	Condition	cpm/mg Dry tissue	p of difference
A	Normal aorta	182.9 ± 25.5 (8)	A–B = ns
B	Normal aorta plus insulin	192.5 ± 15.7 (8)	A–C = ns
C	Diabetic aorta	163.7 ± 32.5 (8)	A–D = <.05
D	Diabetic aorta plus insulin	122.5 ± 7.2 (8)	C–D = <.10

[a] Results expressed as mean ± SEM; glucose = 200 mg/100 ml; insulin = 20 μg/ml; number of observations in parentheses.

After incubation, the tissue was washed in three successive portions of 0.026 *M* Na_2SO_4 and subsequently treated as previously described for isolation of the mucopolysaccharide fraction (3). Uronic acid content was measured with napthoresorcinol (13), and radioactivity was measured in a liquid-scintillation counter.

Results. The addition of insulin (20 μg/ml) to a simple buffer medium containing 200 mg/100 ml glucose had no effect on ^{14}C-acetate uptake by aortas from normal rats. Aortas from diabetic rats, incubated with glucose but in the absence of insulin, showed normal ^{14}C-acetate uptake; the addition of insulin to the incubation medium significantly decreased ^{14}C-acetate uptake into mucopolysaccharides in diabetic aortas (Table I).

Insulin *in vitro* did not influence ^{35}S-sulfate uptake or incorporation in aortas from normal rats. While diabetic aortas showed normal values for sulfate uptake *in vitro* in the basal glucose-containing medium, the addition of insulin produced a significant stimulation of sulfate uptake and incorporation (Tables II and III).

Discussion. The normal ^{14}C-acetate uptake *in vitro* seem in diabetes indicates that the synthesis of glucose-derived moieties of mucopolysaccharides is insulin independent, and that the general depression of carbohydrate metabolism that occurs in diabetes does not affect the synthesis of these substances. Since the addition of insulin depressed uptake to below normal values in diabetic aortas, it would appear that under these conditions, the labeled precursor is shunted to insulin-dependent pathways. The absence of effect of insulin on ^{14}C-acetate uptake into mucopoly-

TABLE II. ^{35}S-Sulfate Uptake *in Vitro* into Aortic Mucopolysaccharides.[a]

Group	Condition	cpm/mg Dry tissue	p of difference
A	Normal aorta	76.9 ± 11.3 (11)	A–B = ns
B	Normal aorta plus insulin	75.6 ± 17.4 (10)	A–C = ns
C	Diabetic aorta	98.1 ± 20.4 (11)	A–D = <.05
D	Diabetic aorta plus insulin	137.6 ± 28.8 (11)	C–D = <.10

[a] Results expressed as mean ± SEM; glucose = 200 mg/100 ml; insulin = 20 μg/ml; 24-hr incubation; number of observations in parentheses.

TABLE III. Specific Activity of ^{35}S-Sulfate Incorporated *in Vitro* into Rat Aortic Mucopolysaccharides.[a]

Group	Condition	cpm/μg Uronic acid	p of difference
A	Normal aorta	4.93 ± 2.06 (11)	A–B = ns
B	Normal aorta plus insulin	4.72 ± 1.50 (11)	A–C = ns
C	Diabetic aorta	4.43 ± 0.82 (10)	A–D = <.01
D	Diabetic aorta plus insulin	8.10 ± 2.42 (11)	C–D = <.01

[a] Results expressed as mean ± SEM; conditions as in Table II; number of observations in parentheses.

saccharides in normal aortas supports the idea that insulin does not influence uronic acid or hexosamine synthesis in a specific manner.

The finding that insulin stimulated the incorporation of ^{35}S-sulfate into mucopolysaccharides of diabetic aortas suggests that it is more specifically involved in the process of sulfation of these substances. The fact that insulin added *in vitro* had no effect on ^{35}S-sulfate incorporation in normal aortas lends support to the hypothesis that there is a complex interrelationship between various factors governing sulfate metabolism in the normal animal, and that one of the involved factors will exert a more significant effect in the absence of another. Growth hormone is known to profoundly influence sulfate metabolism (14–16), and many interactions between insulin and growth hormones *in vivo* have been described (17, 18). In a previous communication, we reported that either pancreatectomy or hypophysectomy produced a pattern of aortic sulfate uptake *in vivo* that was characterized by a peak at 4 hr after injection, and that this finding disappeared in hypophysectomized-pancreatectomized animals (12). We suggested that insulin and growth hormone might act at a similar metabolic site concerned with sulfation in vascular connective tissue, and in the absence of one hormone the action of the other produces a pattern of excessive sulfation. The present results help explain some of the discrepancies between the results of Salmon and Daughaday and those of Wettenhall *et al.* when interpreted from the point of view that only in the absence of one of these hormones will the other significantly stimulate sulfaction. Aortas from diabetic animals, removed from the *in vivo* environment in which both insulin and growth hormone are deficient (the secretion of the latter may be depressed by chronic hyperglycemia), can thus respond *in vitro* to insulin stimulation of sulfation.

Summary. Insulin *in vitro* failed to stimulate the uptake of ^{14}C-acetate or ^{35}S-sulfate into aortic mucopolysaccharides of normal animals. Aortas from diabetic animals showed normal ^{14}C-acetate uptake which was decreased by the addition of insulin, and a normal ^{35}S-sulfate uptake, which was significantly increased by insulin added *in vitro*.

The technical assistance of Miss Irene Villareal is gratefully acknowledged.

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

2. Spiro, R. G., Diabetes **12**, 223 (1963).

3. Cohen, M. P., and Foglia, V. G., Proc. Soc. Exp. Biol. Med. **132**, 376 (1969).

4. Cohen, M. P., and Foglia, V. G., Diabetes, In Press (1970).

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

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

7. Kranz, D., Keim, O., Braselmann, H., and Kunz, J., Acta Biol. Med. Germ. **21**, 563 (1968).

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

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

10. Wettenhall, E. H., Schwartz, P. L., and Bornstein, J., Diabetes **18**, 280 (1969).

11. Daughaday, W. H., and Kipnis, D. M., Recent Progr. Horm. Res. **22**, 49 (1966).

12. Cohen, M. P., and Foglia, V. G., Proc. Soc. Exp. Biol. Med. **133**, 1275 (1970).

13. Dische, Z., *in* "Methods in Carbohydrate Chemistry" (R. L. Whistler and M. L. Wolfrom, eds.), Vol. 1, p. 497. Academic Press, New York (1962).

14. Ellis, S., Hubble, J., and Simpson, M. E., Proc. Soc. Exp. Biol. Med. **84**, 603 (1953).

15. Murphy, W. R., Daughaday, W. H., and Hartnett, C., J. Lab. Clin. Med. **47**, 715 (1956).

16. Asboe-Hansen, G., *in* "Int. Rev. Conn. Tiss. Res." (D. A. Hall, ed.), Vol. II, p. 29. Academic Press, New York (1963).

17. Rabinowitz, D., Merimee, T. J., and Burgess, J. A., Diabetes **15**, 905 (1966).

18. Glick, S. M., Roth, J., Yalow, R. S., and Berson, S. A., Recent Progr. Horm. Res. **21**, 241 (1965).

Received May 11, 1970. P.S.E.B.M., 1970, Vol. 135.