Effect of Pancreatectomy, Hypophysectomy, and Hypophysectomy–Pancreatectomy on Sulfate Uptake in Rat Aortas[1] (34669)

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The role of insulin in sulfation remains disputed. Salmon and Daughaday (1) reported that insulin *in vitro* causes significant stimulation of sulfate uptake by costal cartilage from hypophysectomized rats and from normal fasted rats, whereas Wettenhall *et al.* (2) observed only marginal effects of insulin on ^{35}S sulfate incorporation into growing bones in a steady state culture system. It has been reported that the incorporation of radiosulfate into aortic mucopolysaccharides of diabetic animals is increased 4 hours (3) and depressed 24 hr (4) after the administration of radiosulfate, that the uptake of labeled sulfate in the skin of alloxan diabetic animals is reduced, and that insulin treatment restores the values to normal (5).

It is generally accepted that growth hormone profoundly influences sulfate metabolism (6–8), probably by stimulating the production of a serum suflation factor (1, 9). Insulin added *in vitro* may simulate the *in vitro* effects of this growth hormone dependent sulfation factor (1, 10). Numerous interrelationships between growth hormone and insulin *in vivo* are now recognized (11, 12), and the control of sulfate uptake may be another of these interactions. In a previous communication, we reported that the incorporation of sulfate^{35} into aortic mucopolysaccharides was markedly increased 4 hr after an intraperitoneal injection of (^{35}S) Na_2SO_4 and we suggested that this finding might result from an unopposed action of growth hormone in the pancreatectomized animal (3). Therefore, the present study was undertaken to further delineate the cause of this early sulfate peak. Animals were studied at varying periods of time following pancreatectomy to determine whether or not this peak was dependent on the presence of hyperglycemia, and to determine the effect of hypophysectomy, and of hypophysectomy plus pancreatectomy on the uptake and incorporation of radioactive sulfate into the mucopolysaccharide fraction of rat aortas after an intraperitoneal injection of (^{35}S) Na_2 (SO_4).

Materials and Methods. Male white rats (Institute strain) were used for all studies. 95% pancreatectomy was performed on animals weighing 80–120 g, and the development of diabetes was followed by blood sugar levels after 7-hr fasting. Animals were sacrificed 1, 3, and 5 months postpancreatectomy, and were matched with control animals of the same age and sex.

Hypophysectomy was performed by the parapharyngeal approach on animals weighing 180–220 g. Previously pancreatectomized animals were hypophysectomized approximately 3 months after pancreatectomy. Experiments were conducted 10–30 days after hypophysectomy, using animals of the same age and sex as controls. Hypophysectomized animals in which the gonads failed to become atrophic were discarded.

Animals were sacrificed 2, 4, and 6 hr after an intraperitoneal injection of 50 μCi of (^{35}S) Na_2SO_4 (sp act, 423 mCi/mmole). Aortas were excised and treated as previously described (3), and the mucopolysaccharide fraction, isolated by proteolytic digestion and extensive dialysis (13), was lyophilized. Following reconstitution in 1.0 ml of *n*-propanol:1% cetylpyridinium chloride (2:1), aliquots were removed for determination of uronic acid by the method of Dische (14), and of radioactivity in a liquid scintillation counter.

Results. (a) Effect of pancreatectomy. In

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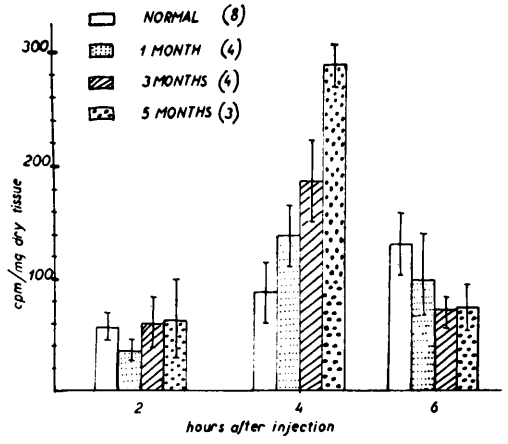

FIG. 1. Sulfate[35] uptake in aortic mucopolysaccharides in normal and partially pancreatectomized rats: results expressed as mean ± SEM; number of animals given in parentheses; mean blood sugar of the various groups mg/100 ml: normal, 113; 1 month, 117; 3 months, 152; 5 months, 200.

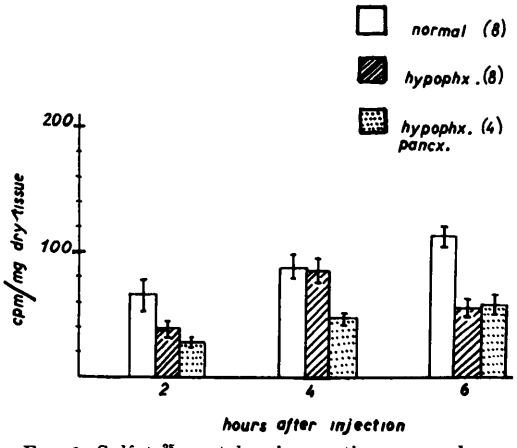

FIG. 2. Sulfate[35] uptake in aortic mucopolysaccharides in normal, hypophysectomized, and hypophysectomized–pancreatectomized rats: results expressed as mean ± SEM; number of animals given in parentheses.

normal animals, sulfate uptake steadily increased over the 6-hr period; in the pancreatectomized animals, there was a consistent increase in ^{35}S sulfate uptake into aortic mucopolysaccharides at 4 hr; whereas, at 6 hr, sulfate uptake was uniformly depressed, in relation to both the 4-hr values as well as the 6-hr control values. The 4-hr peak was seen in all pancreatectomized groups, regardless of the blood sugar, and the height of the peak rose consistently with increasing duration of diabetes. These results are shown in Fig. 1.

(*b*) *Effect of hypophysectomy.* Although the level of (^{35}S) sulfate uptake 4 hr after injection in hypophysectomized rats was the same as that noted in normal animals, the pattern of radioactive sulfate uptake over the 6-hr period studied was distinctly different. Sulfate uptake was highest at 4 hr, and had declined by 6 hr in the hypophysectomized animals, thus approximating the pattern of radiosulfate uptake seen in pancreatectomized animals (Fig. 2). Sulfate incorporation (cpm/μg uronic acid) was greatly increased at 4 hr in the hypophysectomized animals, and had declined by 6 hr, although not to normal levels (Fig. 3).

(*c*) *Effect of hypophysectomy–pancreatectomy.* In animals which were both hypophysectomized and pancreatectomized, the radiosulfate uptake returned to a regular pattern of steady increase during the time period studied, except that the values were lower than normal (Fig. 2). Sulfate incorporation (cpm/μg uronic acid) also followed a normal pattern in the hypophysectomized–pancreatectomized animals, with the distinct absence of a 4-hr peak (Fig. 3).

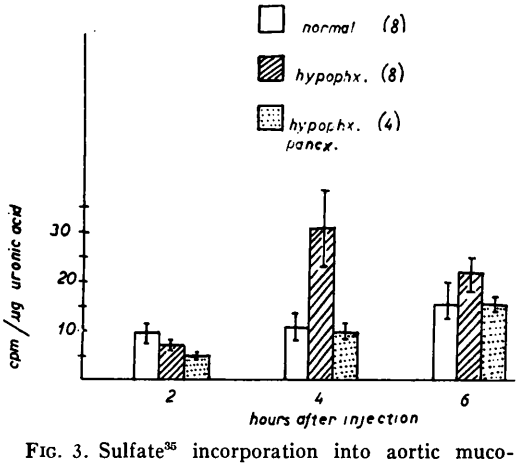

FIG. 3. Sulfate[35] incorporation into aortic mucopolysaccharides in normal, hypophysectomized, and hypophysectomized-pancreatectomized rats: results expressed as mean ± SEM; number of animals given in parentheses.

(*d*) *Uronic acid concentrations.* The concentration of uronic acid was not significantly altered from normal values (mean ± SEM = 6.36 ± 0.86 μg / mg of dry tissue) in the hypophysectomized, early pancreatectomized (less than 3 months since operation), or hypophysectomized–pancreatectomized animals. Uronic acid concentration was significantly altered only in the late-pancreatectomized (5 months since operation) animals (3.52 ± 0.63; $p < .05$).

Discussion. The persistent 4-hr peak in aortic (^{35}S) sulfate uptake seen in the pancreatectomized animals suggests a transient, but intense increase of sulfate uptake which becomes more pronounced with the development of diabetes. Although the blood sugar level remains normal during the prediabetic phase in the pancreatectomized rat (15), serum insulin levels gradually diminish, finally resulting in overt hyperglycemia (16). It would therefore appear that a relative lack, but not necessarily complete absence, of insulin is involved in the appearance of this 4-hr peak rather than the simple presence of hyperglycemia.

The pattern of radiosulfate uptake in the hypophysectomized animals was similar to, although not as striking as that seen in pancreatectomized animals. Thus either a relative lack of insulin in the presence of normal pituitary activity, or absence of pituitary in the presence of normal pancreatic activity, produces a pattern of aortic sulfate uptake that is characterized by a 4-hr peak. In hypophysectomized–pancreatectomized rats, this 4-hr peak disappears completely.

Growth hormone has "insulin like" (17, 18) as well as "contra-insulin" (19, 20) effects, and it has even been proposed that there may be two growth hormones to account for this dichotomy (21). Rabinowitz *et al.* (12) however, have suggested that growth hormone is a single hormone whose insulin-like effects are optimized by the presence of insulin, and whose diabetogenic or contra-insulin effects predominate in the absence of insulin. Our results suggest that both insulin and growth hormone might act at similar metabolic sites concerned with sulfation in vascular connective tissue. As the insulin level progressively diminishes following pancreatectomy, the competitive or regulatory action of insulin on sulfation gradually disappears, producing a pattern of excessive sulfation. In a similar fashion, insulin would have an unopposed action in the hypophysectomized animal. In the absence of both hormones, the pattern of sulfation returns to normal since either source of unopposed stimulation has been removed. We recognize the possibility that the absence of other pituitary hormones might have affected the results observed in the hypophysectomized animals, and further studies are in progress to resolve this question.

It is interesting to note that, while sulfate uptake in the doubly operated animals was depressed, incorporation of sulfate into uronic acid was almost normal. These animals were not yet markedly hyperglycemic (mean blood sugar 150 mg/100 ml), and it is probable that low levels of circulating insulin were still present which were able to maintain sulfate incorporation. In the pancreatectomized animals, striking peaks in both ^{35}S uptake and incorporation were seen; whereas, in the hypophysectomized animals incorporation was more markedly altered, suggesting that insulin has a more potent effect on this aspect of sulfate metabolism.

Summary. The effect of partial pancreatectomy, hypophysectomy, and hypophysectomy plus pancreatectomy on the incorporation of ^{35}S sulfate into the aortic mucopolysaccharides of rats was studied. There was a marked peak in sulfate^{35} uptake 4 hr after the injection of the label in pancreatectomized and in hypophysectomized animals, and the appearance of this peak preceded the development of hyperglycemia in the pancreatectomized animals. In hypophysectomized–pancreatectomized animals, this peak was abolished. The findings suggest that insulin has an important role *in vivo* in the regulation of sulfate metabolism in vascular connective tissue. The results are discussed in relation to a possible interraction between growth hormone and insulin in the regulation of sulfate uptake and incorporation.

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