Effects of Insulin and Glucose on Utilization of Fructose by Isolated Rat Diaphragm.* (20349)

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Results of studies by Price *et al.*(1) and Colowick *et al.*(2) suggested that pituitary and adrenal cortical extracts inhibited hexokinase activity in muscle extracts from alloxan-diabetic rats, and that hexokinase activity was restored by addition of insulin. Experimental results were not consistent, since inhibition of hexokinase activity by adrenal cortical extract with restoration of activity by insulin was obtained in only 50% of the experiments performed. In similar experiments reported by Reid *et al.*(3) and Broh-Kahn and Mirsky(4) the findings were again inconstant. Stadie *et al.*(5,6) found no significant effect of insulin or adrenal cortical extract on hexokinase activity in muscle extracts from normal and diabetic rats. Their findings suggested that pituitary extracts impaired the ability of normal rat-diaphragm to combine with insulin, and that the diaphragms from alloxan-diabetic rats did not combine well with insulin. More recently, however, Chernick *et al.*(7,8) have shown that in the alloxan-diabetic rat liver glucose utilization is initially impeded at the hexokinase stage, and Stadie *et al.*(9,10) demonstrated that in rat-diaphragm the acceleration of glycogen synthesis per μg of bound insulin is greater after removal of the pituitary. Results of experiments presented here suggest that in isolated rat-diaphragms insulin increases the utilization of glucose and fructose by accelerating their phosphorylation by hexokinase.

Methods. Adult white rats fasted 24 hours were decapitated and the diaphragms were removed with minimum trauma(11). Each hemidiaphragm was weighed without preliminary washing and placed directly into the medium contained in a small Erlenmeyer flask. After removal of samples for analysis the 2 flasks (a) and (b) were stoppered and incubated with shaking at 37°C. Samples were removed from the flasks for analysis at the termination of the experiments. The composition of the medium in the 2 flasks was identical except that insulin was added to flask (a), 0.5 unit per cc medium. The medium consisted of 0.1 M phosphate (K_2HPO_4-NaH_2PO_4 mixed in proportion to give a pH of 7.6), $MgCl_2$ 0.005 M, $CaCl_2$ 0.001 M, in final volume 4 cc after addition of fructose and/or glucose. Amorphous insulin (W-1302, 19 units/mg) was obtained from the Eli Lilly Co. Fructose was determined by modifications of the methods of Roe(12) and Nelson (13) using filtrates deproteinized with $ZnSO_4$ and $Ba(OH)_2$. The Nelson method(13) was used for the determination of glucose. pH was determined by means of a Cambridge Research model pH meter with glass electrode.

Results and discussion. Table I presents data obtained in paired experiments on rat diaphragm incubated with and without insulin with combinations of fructose and glucose as substrate. Results of preliminary experiments showed that insulin increased the utilization of glucose by rat diaphragm, in accord with work previously published by Stadie and Zapp(11). In a previous report(14) we have shown that both hexokinase and fructokinase are present in rat diaphragm. Fructose is not converted to glucose in rat diaphragm since glucose-6-phosphatase is not present in muscle(15,16). Results of previous work(18,19) have shown that the hexokinase but not the fructokinase pathway of fructose phosphorylation (Fig. 1) is almost completely inhibited by the presence of an equimolar concentration of glucose. In the experiments here presented, with fructose alone as substrate insulin increased the utilization of fructose by rat diaphragm significantly [$p < .01$, determined by the method of analysis of variance(17)]. However, when both fructose and glucose were

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TABLE I. Results of Experiments on Rat Diaphragm Incubated with and without Insulin with Combinations of Fructose and Glucose as Substrate. 15 experiments.

Insulin in medium	Initial conc., % Fructose	Initial conc., % Glucose	Fructose utilized from medium mg/g tissue	Time, hr	Final pH
+	.2	—	3.22	3	7.41
—	.2	—	2.78	3	7.43
+	.2	—	2.88	3	7.40
—	.2	—	2.19	3	7.44
+	.2	—	3.11	3	7.35
—	.2	—	2.60	3	7.35
+	.2	—	4.73	3	7.43
—	.2	—	2.96	3	7.44
+	.2	—	3.44	3	7.41
—	.2	—	2.47	3	7.42
+	.2	—	3.63	3	7.44
—	.2	—	2.77	3	7.47
+	.1	—	1.31	4	7.39
—	.1	—	0.80	4	7.37
+	.1	—	2.00	4	7.38
—	.1	—	1.05	4	7.39
+	.1	—	2.91	4	7.45
—	.1	—	2.00	4	7.45
+	.1	.1	1.63	4	7.30
—	.1	.1	1.55	4	7.32
+	.1	.1	1.82	4	7.27
—	.1	.1	1.78	4	7.31
+	.1	.1	1.97	4	7.32
—	.1	.1	2.02	4	7.35
+	.1	.1	2.01	4	7.31
—	.1	.1	1.87	4	7.38
+	.1	.1	1.71	4	7.25
—	.1	.1	1.87	4	7.29
+	.1	.1	1.71	4	7.28
—	.1	.1	1.69	4	7.31

in the medium insulin did not affect the utilization of fructose. It appears, therefore, that the metabolism of fructose via the fructokinase system is not affected by insulin, and that insulin accelerates the utilization of glucose and fructose by way of the hexokinase pathway. Since the reactions follow common metabolic pathways after the phosphorylation

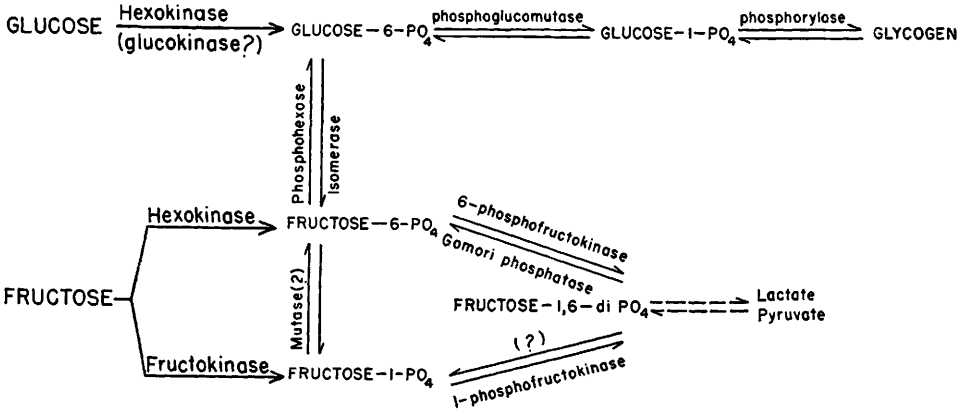

FIG. 1. Pathways for anaerobic metabolism of glucose and fructose in muscle.

of fructose by fructokinase and hexokinase (Fig. 1) it would appear that insulin acts to accelerate the initial phosphorylation of glucose and fructose by hexokinase. It is probable that fructose metabolism in intact animals proceeds almost entirely by way of the fructokinase system since glucose is normally present in tissues *in vivo* in amounts sufficient to inhibit the phosphorylation of fructose by hexokinase. The findings presented in this paper are in agreement with results of experiments *in vivo* by other investigators(7,20-22) suggesting that insulin does not affect the metabolism of fructose in animals and human subjects.

Summary. In isolated rat diaphragm, insulin increased the metabolism of fructose and glucose via the hexokinase system but did not affect the utilization of fructose via the fructokinase pathway. The results suggest that insulin affects the metabolism of glucose and fructose by accelerating their initial phosphorylation by hexokinase.

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Utilization of Sulfur Amino Acids During Healing of Experimental Wounds.* (20350)

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The regeneration of tissue during the healing of wounds has been shown to be markedly accelerated by the addition of methionine to the diet(1-7). More recently, the rate of healing of experimental wounds was shown to be increased to the same extent when equivalent amounts of either cystine or methionine were administered(1). Since the conversion of methionine to cystine is irreversible *in vivo* (9,10), this work may be taken to indicate that cystine is the limiting amino acid in the healing process. The possibility still existed that the sulfur amino acids may not be required *per se*, but rather might serve as a source of sulfur for other compounds required

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