Proceedings of the National Academy of Sciences
Vol. 65, No. 2, pp. 383–387, February 1970

Effects of Glucose on Glycogen Synthetase, Phosphorylase, and Glycogen Deposition in the Perfused Rat Liver*

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Communicated by Earl W. Sutherland, November 10, 1969

Abstract. An increase in the perfusate glucose concentration from near zero to about 11 mM increased glycogen synthesis in the perfused, isolated rat liver from zero to a value about half the maximum seen in the intact animal. Increased synthesis appeared to be due not only to provision of substrate but also to conversion of glycogen synthetase to the active form and of glycogen phosphorylase to the inactive form. These glucose effects, which are apparently independent of changes in levels of hormones or adenosine 3′:5′-cyclic phosphate, may be physiologically significant for control of the blood glucose level.

The liver plays a central role in the regulation of blood glucose.[1, 2] Control appears to be due in part to changes in the tissue level of adenosine 3′:5′-cyclic phosphate (cyclic AMP) resulting from alterations in the activity of the sympathetic nervous system and in the secretion of glucagon and insulin.[3] In addition, the liver possesses intrinsic control systems[2, 4] which help stabilize the blood glucose. One of these is described in this report.

There are several possibilities regarding the nature of these nonhormonal controls. A negative feedback system in which a rise in blood glucose suppresses gluconeogenesis has been proposed by Ruderman and Herrera[5] but these workers could show only a small suppression (25%) of gluconeogenesis from alanine by 17 mM glucose in the isolated, perfused liver, and Exton and Park[6] could not show any inhibition of gluconeogenesis from lactate by 19 mM glucose in a similar system. A quantitatively significant regulation of glucose utilization through adjustments in the rate of hepatic glycolysis also seems unlikely since the intrinsic rate of this process is very low under aerobic conditions.[6] Another possibility, which we have examined in this study, is that glycogen metabolism is affected by the glucose concentration. In this connection, DeWulf and Hers[7] found that intravenous injection of glucose into the mouse led to a rapid activation of hepatic glycogen synthetase and suggested that this effect was independent of changes in insulin or glucocorticoid secretion. Holmes and Mansour[8] reported that glucose inactivated glycogen phosphorylase in the isolated rat diaphragm *in vitro*.

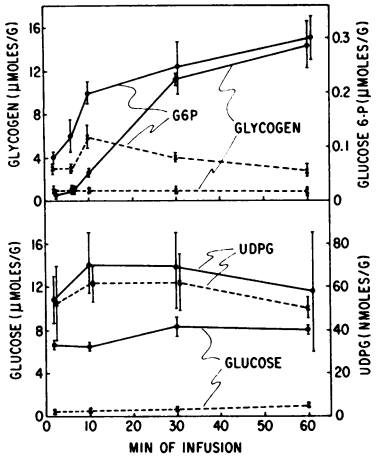
In the present study, we have found that elevation of the glucose concentration in the medium perfusing the isolated rat liver promotes net synthesis of glycogen by conversion of glycogen synthetase to the active form[9] and transformation of glycogen phosphorylase to the inactive form. These effects of glucose appear to be independent of changes in the tissue level of cyclic AMP.

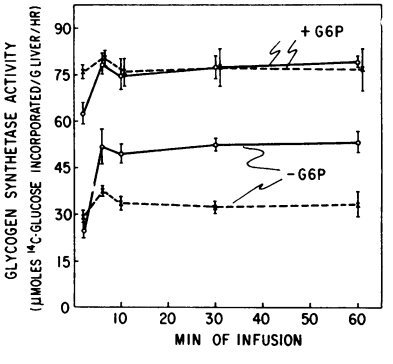
Materials and Methods. Male Sprague-Dawley rats weighing 100–140 gm and fasted 18–22 hr were used. The technique of liver perfusion and the methods of glucose and glycogen analysis have previously been described in detail.[6] Livers were perfused at 37° with oxygenated Krebs-Henseleit bicarbonate buffer, pH 7.4, containing 3% bovine serum albumin and 20% bovine erythrocytes. Tissue samples were rapidly removed and frozen[10] at appropriate times. Glycogen synthetase was assayed by the method of Thomas, Schlender, and Larner[11] and phosphorylase according to the technique of Cornblath and co-workers.[12] Glucose, glucose-6-P and UDPG were measured in perchlorate extracts of frozen tissue by enzymatic methods.[13] We are grateful to Dr. G. A. Robison for determinations of adenosine 3′:5′-phosphate.[14] Glucagon was kindly supplied by Eli Lilly Co.

Results. The time course of glycogen deposition was examined in livers from fasted rats. Livers were first perfused in a recirculating system for 30 minutes with glucose-free medium in order to minimize the effects of hormonal factors carried over from the intact animal. The test perfusion was then begun as described in Figure 1 without or with 11 mM glucose (200 mg %) in the medium.

Glycogen remained at a low level in the controls but increased progressively in tissue perfused with glucose (Fig. 1). The level of glucose-6-P rose in parallel with that of glycogen but the concentration of UDPG was not affected. The free glucose content of the tissue was high from the start in the glucose perfused tissue as expected since glucose equilibrates very rapidly across the plasma membrane of hepatic cells.[15]

The *a* form of glycogen synthetase (active without added G-6-P) rose markedly within six minutes after beginning the infusion of glucose and remained elevated thereafter (Fig. 2). Total synthetase activity did not change.[16]





(*Left*) FIG. 1.—Time course of the effects of glucose infusion on the levels of glycogen, glucose-6-P, UDPG, and glucose in perfused livers from fasted rats. Livers were perfused with recirculating medium for 30 min and then the perfusate was replaced with one containing either 11 mM or no glucose. Tissue samples were frozen at the designated times after the change to fresh medium. Glycogen values are given in glucose equivalents. x—x control livers; ●——● glucose infused livers.

(*Right*) FIG. 2.—Time course of the activation of glycogen synthetase by glucose infusion. Experimental details were as for Fig. 1.

Glucose infusion also produced a substantial inactivation of phosphorylase (measured in the presence of excess AMP) which was evident within two minutes of exposure to the sugar (Table 1).

Since changes in the activity of glycogen synthetase and phosphorylase can be caused by variations in the tissue level of cyclic AMP, it was important to determine whether glucose infusion lowered the level of this compound. As we have stressed earlier,[17] it is difficult to detect reductions in tissue cyclic AMP levels below the control values. The effects of glucose were therefore examined in the presence of a suboptimal, presumably physiological, level of glucagon which caused a small rise in tissue cyclic AMP and activated phosphorylase submaximally. Table 2 shows that glucose counteracted the phosphorylase activation but did not reduce the level of cyclic AMP.

TABLE 1. *Effect of glucose on phosphorylase activity.*

Infusion	Duration of infusion (min)	Number	Phosphorylase activity (μg P_i released/gm liver/30 min)
Saline	2	4	1624 ± 146
Glucose	2	4	859 ± 60
Saline	60	4	1375 ± 31
Glucose	60	4	856 ± 61

Experimental details were as for Fig. 1.

TABLE 2. *Effect of glucose on glucagon-induced activation of phosphorylase and accumulation of cyclic AMP.*

Glucagon concentration	Glucose concentration (mM)	Number	Phosphorylase activity (μg P_i released/gm liver/30 min)	Cyclic AMP (nmoles/gm)
0	1.2	4	941 ± 44	0.56 ± 0.02
1×10^{-10} *M*	1.3	6	1831 ± 94	0.73 ± 0.02
1×10^{-10} *M*	10.4	6	1278 ± 54	0.74 ± 0.01

Livers from fasted rats were perfused for 60 min with or without glucose at 10 mM initial concentration. An infusion of glucagon or saline was then started and livers were frozen 10 min later.

Discussion. The present results show that a high glucose concentration can increase the net rate of glycogen synthesis in the perfused liver from 0 to about 25 μmoles of hexose equivalents per gram of liver per hour (corresponding to a gain of 0.5 per cent wet weight of liver per hour). Since this rate is already about half the maximum observed *in vivo*,[18] it appears that glucose can be a very substantial stimulus to glycogen deposition even in the absence of facilitating hormonal influences.

The following considerations suggest that this glucose effect could by physiologically important: (1) The increase in glycogen synthetase a (Fig. 2) should promote glucose utilization and glycogen synthesis. Presumably only the *a* form has significant activity *in vivo* since the K_m value[9] of the *b* form for UPDG (>2 mM with <1 mM G-6-P) and the K_a value[9] for G-6-P (2 mM) are far above the mean concentrations of UPDG (0.1 mM) and G-6-P (0.1–0.6 mM) in the absence or presence of glucose. The rise in G-6-P level with glucose should have only a minor reinforcing action on the stimulatory effect of the enzyme conversion

in view of the low K_a of the *a* form for G-6-P (0.06 mM). (2) The reduction in total phosphorylase activity (about 40% in Table 1) should slow glycogenolysis and glucose production. Presumably, the enzyme is converted to the inactive, dephosphorylated form[19] analogous to the *b* form of the synthetase. (3) Glucose can counteract the phosphorylase activating effect of a physiological level of glucagon. Since insulin also has this effect[17] it may be an effective synergist with glucose in the intact animal, where the plasma levels of both substances usually rise and fall in parallel. (4) The onset of the glucose effect is so rapid that it could be effective in the minute-to-minute control of blood glucose levels. (5) Experiments of DeWulf and Hers[7, 20] suggest that the glucose effect, which they observed and which is presumably the same as seen in these experiments, takes effect only when the concentration of the blood glucose rises above the normal postabsorptive level of about 5 mM.

The increased availability of glucose as a substrate undoubtedly plays an important role in promoting glycogen synthesis. It is of interest, however, that the level of the precursor substrate, UDPG, was not increased at the high glucose concentration. Presumably, the greater rate of UDPG utilization consequent to synthetase activation keeps pace with the increase in UDPG formation.

The present glucose effects are consistent with the findings: (1) that intravenous glucose in intact mice activates glycogen synthetase by a mechanism independent of insulin secretion,[7, 20] and (2), that glucose inactivates phosphorylase in the rat diaphragm.[8] The observation of Holmes and Mansour[21] that glucose activates phosphorylase phosphatase is of great interest and analogous activations may underlie the present enzyme interconversions. It would seem unlikely that a reduction in the tissue level of cyclic AMP is involved. A change in tissue glucose-6-P is also probably not responsible since phosphorylase was maximally inhibited after 2 minutes of glucose infusion even though glucose-6-P was not increased (Fig. 1). The studies of Maddaiah and Madsen[22, 23] also render it unlikely that this metabolite exerts significant control on phosphorylase under physiological conditions. The possibility that glucose inhibition of phosphorylase activation by glucagon is due to increased glucose-6-P cannot be as readily excluded. Glucose-6-P inhibits the cyclic AMP-induced activation of dog liver phosphorylase by 74 per cent at 0.8 mM and by 21 per cent at 0.08 mM.[24] It is therefore possible that the increased intracellular concentration of this metabolite in the glucose perfused livers (0.35 mM) was partly responsible for the inhibition observed.

The glucose effect may account for the unexpectedly small activation of liver phosphorylase by glucagon observed by Glinsmann and Mortimore[25] when measurements were made at times of high glucose concentration. The inability of these workers to demonstrate an insulin antagonism on the activation of phosphorylase by glucagon might similarly be attributed to the marked decrease in glucose concentration caused by the insulin.

We wish to thank Dr. G. A. Robison for performing the cyclic AMP assays. We are also grateful to Mr. J. Parke Brineaux for his expert technical assistance and to Drs. E. W. Sutherland and J. G. Hardman for their helpful comments.

* This work was supported by program project grant AM-07462 from the National Institutes of Health, U.S. Public Health Service.

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[16] A carryover of tissue G-6-P in the assay should not have seriously affected the estimate of synthetase *a*. At 10 min, for example, when synthetase appeared to be already at a maximum, the quantity of G-6-P carried over would give a final concentration in the assay that was an order of magnitude below the K_m for the *a* form.[9] Furthermore, synthetase *a* did not increase significantly at later time intervals although the carryover was at least doubled.

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