GLYCOGEN SYNTHETASE AND CONTROL OF GLYCOGEN SYNTHESIS IN MUSCLE*

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Glycogen synthetase catalyzes the reaction

$$\text{uridinediphosphate glucose} + \text{glycogen}_{(n)} \rightarrow \text{uridinediphosphate} + \text{glycogen}_{(n+1)}$$

In skeletal muscle glycogen synthetase exists in two enzymatically interconvertible forms: synthetase I, active in the absence of glucose-6-phosphate, and synthetase D, active in the presence of glucose-6-phosphate, but showing greatly reduced activity in its absence (Rosell-Perez, et al., 1962; Traut and Lipmann, 1963).

This enzyme system is well suited to be an important regulator of glycogen synthesis (Leloir and Cardini, 1962; Villar-Palasi and Larner, 1961). A study of the relative amounts of each form of the enzyme in skeletal muscle of anesthetized living mice under differing physiological situations has been reported (Danforth, 1964). It was noted that, when glycogen levels were varied by stimulation, an inverse relationship existed between tissue concentrations of glycogen and the fraction of synthetase in the I form, i.e. (synthetase activity in the absence of glucose-6-phosphate)/(synthetase activity in the

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presence of 10^{-2} M glucose-6-phosphate). (See Fig. 1.) The fact that more synthetase was present in the I form when the tissue concentrations of glycogen were low suggested that a control mechanism might be operating to regulate the glycogen stores; however, the possibility that this relationship was coincidental and that muscle contraction affected both the glycogen levels and the glycogen synthetase by separate mechanisms was not excluded.

A similar relationship between glycogen level and synthetase I activity

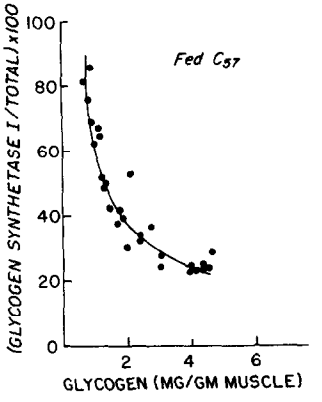

Fig. 1. Relationship between glycogen synthetase I and glycogen in leg muscles of anesthetized living mice. The tissue glycogen was varied by previous muscle contraction. The leg was quick frozen by clamping with Wollenberger tongs pre-cooled in liquid nitrogen. Part of the muscle was analyzed for glycogen (Pfleiderer, 1963) after extraction with hot KOH. Another part was powdered while frozen and extracted at 0° with Tris-acetate 50 mM (pH 7.8), EDTA 4 mM, in the presence of 4 mg of charcoal per 100 mg muscle. The volume was calculated to give a 50:1 dilution of the muscle (w/v). The debris was removed by centrifugation in the cold at 4000 x g. The extract was then assayed for synthetase activity by the method of Kornfeld and Brown (1962) in the absence of added glucose-6-phosphate and in the presence of 10^{-2} M glucose-6-phosphate. The concentration of endogenous glucose-6-phosphate in the reaction mixture was less than 1 per cent of the apparent K_m value ascertained for activation of synthetase D.

has now been found in the isolated "intact" rat diaphragm. (Fig. 2) . In these experiments diaphragms with high glycogen levels were obtained by loading the animals with 450 mg glucose by intravenous injection 30 to 60 min prior to death. Glycogen depleted diaphragms were obtained from animals injected with 0.25 mg epinephrine antemorten and/or rendered hypoxic for 5 min before death by increasing the concentration of N_2 in the inspired air. It may be seen that insulin acts to shift the curve to the right without disturbing the basic relationship between glycogen and synthetase I. These data confirm the report of Larner, et al., (1961) that insulin acts to increase the amount of synthetase I in the isolated rat diaphragm and further define this effect. We have also confirmed that the presence of glucose in the medium is not necessar for this action of insulin.

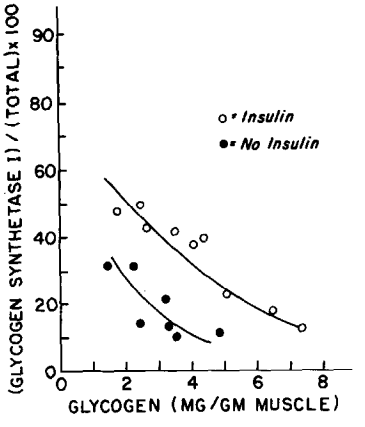

Fig. 2. Relationship between glycogen synthetase I and glycogen in isolated rat diaphragm. Intact diaphragms such as those described by Kipnis and Cori (1957) were incubated with shaking for 15 min at 37° in 150 ml beakers containing 60 ml Ringer's bicarbonate solution gassed with 95% O_2 - 5% CO_2. Each diaphragm was then transferred to a second beaker containing 40 ml Ringer's solution with 10 mM glucose with or without insulin 0.2 u per ml. After 30 min the diaphragm was frozen in liquid N_2. One hemidiaphragm was assayed for glycogen after KOH extraction; the other hemidiaphragm was powdered while frozen and then extracted at 0° in Tris-acetate 50 mM (pH 7.8), EDTA 4 mM, NaF 20 mM. Further procedures are described in thelegend to Fig. 1. Glycogen is expressed per g tissue wet weight.

Thus the inverse relationship between glycogen level and the relative activity of synthetase I is present in the anesthetized mouse and in the isolated rat diaphragm and remains present whether glycogen is depleted by stimulation, epinephrine, or hypoxia, or is elevated by glucose loading. It therefore seems safe to assume that the amount of muscle glycogen acts in an as yet undefined fashion to regulate the interconversion of the synthetase system and thus determines the fraction of synthetase in the I form. In order to support the concept that this is an important mechanism for controlling tissue glycogen levels, it remains to be shown that the relative activity of synthetase I is related to the rate of glycogen synthesis. Experiments demonstrating this relationship in insulinized rat diaphragms are illustrated in Fig. 3.

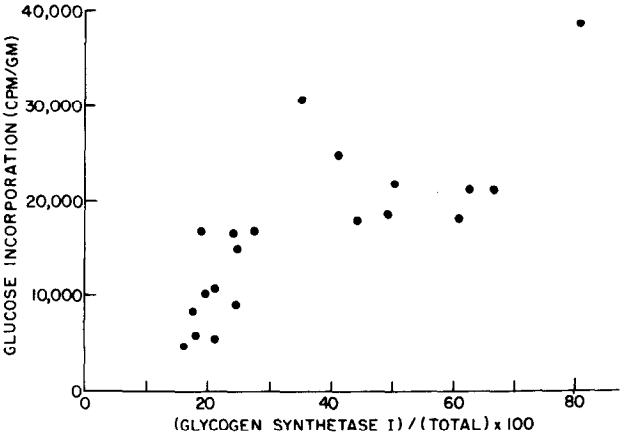

Fig. 3. Relationship between synthetase I activity and incorporation of glucose-u-C^{14} into glycogen of rat diaphragm. Intact diaphragms were placed in 37° aerobic Ringer's bicarbonate solution for 15 min and subsequently incubated aerobically with shaking for 40 min in the presence of insulin 0.2 u per ml. Glucose-u-C^{14} (specific activity 3.01×10^7 cpm per mmole) was added to a concentration of 5 mM during the last 10 min of incubation. One hemidiaphragm was assayed for synthetase I after freezing and extraction as described in the legend of Fig. 2. The glycogen from the other hemidiaphragm was extracted in hot KOH, precipitated with ethanol and then washed twice with 95% ethanol. The precipitate was redissolved in H_2O, plated and counted with a windowless gas flow counter at infinite thinness checked by counting different amounts of each sample. Radioactivity is expressed as cpm per g tissue wet weight.

The data here reported establish on reasonably firm grounds the hypothesis that the interconversion of the two forms regulates the rate of glycogen synthesis in the insulinized rat diaphragm in vitro. In turn the fraction of enzyme in the I form is related to tissue levels of glycogen in such a way as to promote glycogen synthesis when the glycogen stores are depleted and to slow glycogen synthesis when the glycogen stores are plentiful. The direct action of insulin to stimulate glycogen synthesis (Larner, et al., 1959) results not only from promotion of glucose transport across the cell membrane (Morgan, et al., 1961) but also by an independent action that affects the regulation of the synthetase system by glycogen.

The mechanism by which the relative proportion of the two interconvertible forms of the synthetase is affected by the amount of glycogen in the cell is a matter of speculation. In general, it must affect the action of one or both of the converting enzymes. It seems quite plausible that such an effect might be related to tight binding of glycogen to the synthetase thus affecting the accessibility of the protein to the converting enzymes. Work along this line is in progress.

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