Dephosphorylation and Activation of Exogenous Glycogen Synthase by Adipose-Tissue Phosphatase

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(Received 28 November 1979)

Exogenous purified rabbit skeletal-muscle glycogen synthase was used as a substrate for adipose-tissue phosphoprotein phosphatase from fed and starved rats in order to (1) compare the relationship between phosphate released from, and the kinetic changes imparted to, the substrate and (2) ascertain if decreases in adipose-tissue phosphatase activity account for the apparent decreased activation of endogenous glycogen synthase from starved as compared with fed rats. Muscle glycogen synthase was phosphorylated with [γ - 32 P]ATP and cyclic AMP-dependent protein kinase alone, or in combination with a cyclic AMP-independent protein kinase, to 1.7 or 3 mol of phosphate per subunit. Adipose-tissue phosphatase activity determined with phosphorylated skeletal-muscle glycogen synthase as substrate was decreased by 35–60% as a consequence of starvation. This decrease in phosphatase activity had little effect on the capacity of adipose-tissue extracts to activate exogenous glycogen synthase (i.e. to increase the glucose 6-phosphate-independent enzyme activity), although there were marked differences in the activation profiles for the two exogenous substrates. Glycogen synthase phosphorylated to 1.7 mol of phosphate per subunit was activated rapidly by adipose-tissue extracts from either fed or starved rats, and activation paralleled enzyme dephosphorylation. Glycogen synthase phosphorylated to 3 mol of phosphate per subunit was activated more slowly and after a lag period, since release of the first mol of phosphate did not increase the glucose 6-phosphate-independent activity of the enzyme. These patterns of enzyme activation were similar to those observed for the endogenous adipose-tissue glycogen synthase(s): the glucose 6-phosphate-independent activity of the endogenous enzyme from fed rats increased rapidly during incubation, whereas that of starved rats, like that of the more highly phosphorylated muscle enzyme, increased only very slowly after a lag period. The observations made here suggest that (1) changes in glucose 6-phosphate-independent glycogen synthase activity are at best only a qualitative measure of phosphoprotein phosphatase activity and (2) the decrease in glycogen synthase phosphatase activity during starvation is not sufficient to explain the differential glycogen synthase activation in adipose tissue from fed and starved rats. However, alterations in the phosphorylation state of glycogen synthase combined with decreased activity of phosphoprotein phosphatase, both as a consequence of starvation, could explain the apparent markedly decreased enzyme activation.

The pronounced changes in adipose-tissue glycogen during cycles of feeding and starving (Shafir *et al.*, 1965; Steinberg *et al.*, 1975) suggested that

Abbreviations used: SDS, sodium dodecyl sulphate; Tes, 2-[[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]amino]-ethanesulphonic acid.

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this paradigm might be useful in elucidating the metabolic control of glycogen synthase activity. Glycogen synthase in rat adipose tissue is highly dependent on glucose 6-phosphate for activity. Steinberg *et al.* (1975) and Eichner (1976) observed that, when aqueous adipose-tissue extracts from fed rats were incubated with Mg^{2+} , the glucose 6-phosphate-independent activity of the endogenous glycogen synthase increased rapidly. In contrast,

glycogen synthase activation in adipose-tissue extracts from starved rats was markedly reduced. These observations suggested that adipose-tissue glycogen synthase phosphatase activity might be depressed during starvation. Similar findings have been made with liver and heart extracts from starved or diabetic rats and were ascribed to decreases in phosphatase activity (Mersmann & Segal, 1969; Gold, 1970; Das, 1973; Tan & Nutall, 1976; Nutall *et al.*, 1976; Eichner, 1976).

Glycogen synthase purified from skeletal muscle as a glucose 6-phosphate-dependent form contains multiple covalently bound phosphate groups (Roach *et al.*, 1976). Glycogen synthase can be phosphorylated *in vitro* by cyclic AMP-dependent and -independent protein kinases to 3 or more mol of phosphate per subunit of enzyme (Soderling, 1975; Huang *et al.*, 1975; Nimmo *et al.*, 1976; Roach *et al.*, 1976; Itarte *et al.*, 1977; Soderling *et al.*, 1977; Brown *et al.*, 1977; Proud *et al.*, 1977). Phosphorylation of specific site(s) on glycogen synthase greatly increases the dependence of enzyme activity on glucose 6-phosphate (Soderling *et al.*, 1977; Proud *et al.*, 1977). Further phosphorylation produces little additional change in glucose 6-phosphate-dependence, but increases the apparent activation constant of the enzyme for glucose 6-phosphate (Nimmo *et al.*, 1976; Brown *et al.*, 1977), and affects the rate of subsequent dephosphorylation by phosphoprotein phosphatase (Proud *et al.*, 1977; Khatra & Soderling, 1978).

Adipose-tissue glycogen synthase from starved rats had a higher activation constant for glucose 6-phosphate than the enzyme from fed rats (Eichner, 1976; Eichner & Kaslow, 1979; Kaslow & Mayer, 1979). We have made similar observations when comparing the kinetic properties of cardiac-muscle glycogen synthase in fed and starved rats (J. H. Brown & S. E. Mayer, unpublished work). It is likely that the phosphorylation state of glycogen synthase is under metabolic control and is determined by the activities of both protein kinases and phosphatases. If the predominant form of the enzyme in tissues from starved animals is more highly phosphorylated, or is phosphorylated at different sites from those on the enzyme of fed rats, interpretation of the defect in enzyme activation during starvation becomes more complex. One must consider not only the possibility that glycogen synthase phosphatase activity is decreased, but also the possibilities that the altered glycogen synthase is a poorer substrate for glycogen synthase phosphatase, or that a different phosphatase is required for activation of the altered substrate. In addition the apparent defect in glycogen synthase activation during starvation need not result from a phosphatase deficiency, but could instead be explained by dephosphorylation of glycogen synthase preferentially at sites that do not

regulate glucose 6-phosphate-independent enzyme activity.

In order to compare glycogen synthase phosphatase activity in adipose tissue from fed and starved rats, we have used exogenous muscle glycogen synthase phosphorylated with [$\gamma^{32}\text{P}$]ATP. Muscle glycogen synthase was phosphorylated to various extents with cyclic AMP-dependent and -independent protein kinases in order to study the substrate specificity of the phosphatase(s) as well as the relationship of synthase dephosphorylation to increases in glucose 6-phosphate-independent enzyme activity.

Experimental

Enzymes

Glycogen synthase was purified from fresh rabbit skeletal muscle by a modification (Brown *et al.*, 1977) of the method of Soderling *et al.* (1970). The enzyme preparation used in this study had a specific activity of approx. 10 units/mg of protein and showed one major band (mol.wt. 89 000) and one faint band (of lower molecular weight) on polyacrylamide-gel electrophoresis in 7.5% SDS. The catalytic subunit of cyclic AMP-dependent protein kinase and a cyclic AMP-independent protein kinase were purified from fresh rabbit skeletal muscle as previously described (Brown *et al.*, 1977).

Glycogen synthase phosphorylation

Purified muscle glycogen synthase was phosphorylated by the catalytic subunit of cyclic AMP-dependent protein kinase alone or in combination with the cyclic AMP-independent protein kinase at 30°C in a reaction volume of 150–300 μl which contained: 0.4–1.0 mg of glycogen synthase/ml, 25 mM-Tes buffer, pH 7.0, 10 mM-magnesium acetate, 30 mM-2-mercaptoethanol, 1 mM- $[\gamma^{32}\text{P}]$ ATP ($1 \times 10^9 - 2 \times 10^9$ c.p.m./ μmol) or unlabelled ATP, and protein kinase. The stoichiometry of phosphorylation of the skeletal-muscle enzyme and the kinetic changes resulting from phosphorylation have been described (Brown *et al.*, 1977). The maximal phosphate incorporation catalysed by the cyclic AMP-dependent protein kinase was 1.7 mol of phosphate per subunit (mol. wt. 90 000) of muscle glycogen synthase. Glycogen synthase phosphorylated with cyclic AMP-dependent protein kinase plus a cyclic AMP-independent protein kinase to about 3 mol of phosphate per subunit had a lower proportion of glucose 6-phosphate-independent activity and a higher activation constant for glucose 6-phosphate than the enzyme phosphorylated with cyclic AMP-dependent protein kinase alone (Brown *et al.*, 1977).

Phosphorylated skeletal-muscle glycogen synthase was separated from $[\text{32P}]$ ATP by column

chromatography on Sephadex G-25 (fine grade). Approx. 300 μ l of the glycogen synthase phosphorylation mixture was chromatographed on a column (50 cm $\times$ 5 mm) of Sephadex G-25 and a Gieger counter used to monitor 32 P in the column eluate. Glycogen synthase was quantitatively recovered in the first 700 μ l eluted from the column with 20 mM-Tris/HCl/1 mM-EDTA/5% sucrose/40 mM-2-mercaptoethanol, pH 7.5.

Glycogen synthase phosphatase assay

Male Sprague-Dawley rats (Hilltop) weighing 120–150 g upon arrival were housed in a light-controlled (12 h dark, 12 h light) animal room and received Purina Rat Chow and water *ad libitum* for 1–3 weeks before initiation of the experiments. When starved rats were used, food was removed 42–45 h before the animals were killed. Animals were stunned and decapitated, and the epididymal fat-pads were dissected and homogenized in 3 vol. of ice-cold 10 mM-Tris/HCl/1 mM-EDTA, pH 7.5, in a Teflon/glass homogenizer. The homogenate was centrifuged at 10 000 g for 20 min, the floating fat layer discarded and the fluid layer (adipose-tissue extract) removed to ice and assayed within 30 min. The terms adipose-tissue extract or fat extract are used to describe the cell-free fluid layer below the floating fat cake in centrifuged aqueous homogenates of epididymal fat-pads. The phosphatase incubation mixture contained 1 part of adipose-tissue extract plus 3 parts of the column eluate containing glycogen synthase, diluted to give a final glycogen synthase activity of 30–50 munits (or approx. 5 μ g) of glycogen synthase/ml.

The phosphatase incubation mixture (final volume 1–3 ml), containing 10 mM-MgCl₂, was incubated at 30°C and three 100 μ l portions were removed at various times for determination of glycogen synthase activity, protein-bound 32 P and trichloroacetic acid-soluble 32 P. Protein-bound 32 P was assayed by spotting samples diluted with carrier protein (bovine serum albumin, 1.5 mg/ml) on Whatman 3 MM paper discs, which were then washed three times (30 min each) in 10% (w/v) trichloroacetic acid, dried and counted for radioactivity in Omnifluor/toluene (4 g/l). For determination of trichloroacetic acid-soluble 32 P, bovine serum albumin and trichloroacetic acid to final concentrations of 1.2 mg/ml and 12% (v/v) respectively were added to the samples removed from the incubation. The samples were left on ice for at least 30 min, centrifuged (10 000 g, 30 min), and the supernatant fractions removed and counted for radioactivity in Omnifluor/toluene containing Triton X-100 (33%, v/v). For assay of glycogen synthase activity, portions were diluted in 4 vol. of 50 mM-Tris/HCl/1 mM-EDTA/20 mM-KF/0.25 M-sucrose/0.1% albumin, pH 7.5. Glycogen synthase was

assayed by the method of Thomas *et al.* (1968), with 4.6 mM-UDP-[U- 14 C]glucose (0.4 Ci/mol) and 50 μ l of enzyme in a final volume of 125 μ l. One unit of activity is that amount that catalysed the incorporation of 1 μ mol of UDP-glucose into glycogen per min. The reaction was linear with time and protein for the 20 min assay period used. Glucose 6-phosphate-independent activity was determined by measurement of activity in the absence of glucose 6-phosphate; total activity was determined in the presence of 7.5 mM-glucose 6-phosphate.

Endogenous glycogen synthase activation was determined on 1:3 (w/v) adipose-tissue extracts prepared in 10 mM-Tris/HCl/1 mM-EDTA, pH 7.5. Extracts were incubated with 10 mM-MgCl₂, samples removed at various times and diluted in 2 vol. of buffer and assayed as described above. The activation constant ($A_{0.5}$) for glucose 6-phosphate was determined with 0.5 mM-UDP-[U- 14 C]glucose (2 Ci/mol), and portions of the incubated extract were diluted in 4 vol. of buffer before assay. The $A_{0.5}$ for glucose 6-phosphate was calculated from Hill plots.

In some experiments the activity of the endogenous adipose tissue glycogen synthase was determined and subtracted from the total activity at all time points measured for the phosphatase assay. The activity contributed by the endogenous glycogen synthase was less than 10% of the total and was not a significant determinant of the glycogen synthase activation pattern.

Protein was determined by the method of Lowry *et al.* (1951).

Materials

ATP (disodium salt), glucose 6-phosphate, UDP-glucose, oyster glycogen and Sephadex G-25 were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A., UDP-[U- 14 C]glucose (200 Ci/mol) was from Amersham-Searle, Arlington Heights, IL, U.S.A., and [γ - 32 P]ATP (10–30 Ci/mmol) was purchased from ICN, Cleveland, OH, U.S.A.

Results

Adipose-tissue extracts were prepared as described in the Experimental section and incubated for various times at 30°C in the presence of 10 mM-MgCl₂. The results confirmed those reported previously (Steinberg *et al.*, 1975; Eichner, 1976). They demonstrated a rapid increase in the proportion of glucose 6-phosphate-independent activity of glycogen synthase from fed rats, in contrast with the very slow increase seen after a lag period in extracts from starved rats (Fig. 1). Total glycogen synthase activity (assayed with 7.5 mM-glucose 6-phosphate) was somewhat more labile under our conditions (30°C) than at 20°C. The reason for the lability of

Fig. 1. *Endogenous glycogen synthase activation in adipose-tissue extracts from fed and starved rats*

Glycogen synthase activity was determined in 1:3 (w/v) aqueous adipose-tissue extracts from fed ($\blacktriangle$) or starved ($\triangle$) rats after incubation at 30°C for the times indicated. Values are averages from two separate experiments. The (averaged) protein concentrations of the fat extracts were 1.7 mg/ml for fed and 3.2 mg/ml for starved rats. Total glycogen synthase activity (assayed with 7.5 mM-glucose 6-phosphate) was 15.7 munits/ml for fed and 15.8 munits/ml for starved rats at zero time; activity decreased gradually to 40% (fed) and 54% (starved) of these values during the 60 min incubation.

Fig. 2. *Dephosphorylation and activation of exogenous glycogen synthase phosphorylated by cyclic AMP-dependent protein kinase*

Glycogen synthase dephosphorylation and glucose 6-phosphate-independent and total enzyme activities were determined within the same experiment on three separate occasions. The values plotted are means $\pm$ S.E.M. for protein-bound phosphate (O, $\bullet$) and glucose 6-phosphate-independent activity (Δ , $\blacktriangle$) expressed as percentage of total activity. Total activity was constant and averaged 39 munits/ml in the three experiments. Adipose-tissue protein concentrations in the incubations were 0.8 and 1.4 mg/ml (average) for extracts from fed and starved rats respectively. $\blacktriangle$, $\bullet$, Fed rats; $\triangle$, O, starved rats.

the adipose tissue enzyme is unclear, although we have subsequently determined that dithiothreitol affords some protection. The glucose 6-phosphate activation constant of glycogen synthase in extracts from fed rats was 0.95 mM, and decreased to less than 70 μ M after 20 min of incubation. The enzyme from starved rats had an activation constant of 1.65 mM; this value decreased to 0.85 and 0.55 mM after 20 and 60 min of incubation respectively.

Exogenous glycogen synthase containing 1.7 mol of phosphate per subunit ($A_{0.5}$, approx. 0.25 mM) was rapidly dephosphorylated by extracts from both fed and starved rats (Fig. 2). Decreases in protein-bound phosphate were quantitatively paralleled by increases in trichloroacetic acid-soluble phosphate (not shown). Glucose 6-phosphate-independent activity increased in parallel with enzyme dephosphorylation. In contrast with the results shown in Fig. 1 with endogenous glycogen synthase as substrate, there was no qualitative difference in activation of the exogenous substrate by adipose-tissue extracts from fed and starved rats.

Muscle glycogen synthase phosphorylated to 3 mol of phosphate per subunit ($A_{0.5}$, approx. 2 mM)

by combining cyclic AMP-dependent and -independent kinases was then tested as a substrate for the adipose-tissue phosphatase (Fig. 3). Dephosphorylation by extracts from either fed or starved rats was initially rapid, and declined progressively after removal of approx. 1 mol of phosphate per subunit (9–12 min). The initial dephosphorylation resulted in little increase in glucose 6-phosphate-independent activity. With extracts from starved rats, a pronounced lag period preceded any measureable increase in glucose 6-phosphate-independent glycogen synthase activity. The time course of enzyme activation by extracts from starved rats was delayed by the longer lag period, which reflected in part the lower initial rate of enzyme dephosphorylation. However, the difference in activation of this exogenous substrate by extracts from fed and starved rats was only slight when compared with the markedly distinct profiles shown in Fig. 1 for the

Fig. 3. Dephosphorylation and activation of exogenous glycogen synthase phosphorylated by cyclic AMP-dependent and -independent protein kinases

The values plotted are means $\pm$ S.E.M. from three experiments in which protein-bound phosphate (O, $\bullet$) and glycogen synthase activity (Δ , $\blacktriangle$) were determined. Total glycogen synthase activity was 42 munits/ml (average) and remained constant. The adipose-tissue protein concentrations in incubations with extracts from fed and starved rats respectively were 0.6 and 0.8 mg/ml (average). $\blacktriangle$, $\bullet$, Fed rats; Δ , O, starved rats.

activation of endogenous adipose-tissue glycogen synthase.

Adipose-tissue phosphatase activity was also measured at a higher substrate concentration, since it was possible that greater differences between fed and starved animals would be seen at saturating concentrations of glycogen synthase (Fig. 4). Muscle synthase phosphorylated to approx. 3 mol per subunit was used as the substrate at a concentration similar to that used in the experiments shown in Fig. 3 (5 μ g/ml) and at a 10-fold higher concentration. The initial rates of glycogen synthase dephosphorylation were determined from the data shown in Fig. 4, where phosphatase activity is expressed as pmol of phosphate released per mg of adipose-tissue protein. Phosphatase activity at the higher substrate concentration was five times that at the lower concentration. However, at either substrate concentration, the phosphatase activity in adipose tissue of starved rats was approx. 65% of that in fed rats.

A short lag period preceded activation of the endogenous glycogen synthase in adipose tissue from starved rats (Fig. 1). A lag period also preceded the increase in glucose 6-phosphate-independent activity of muscle glycogen synthase phosphorylated to 3 mol per subunit (Fig. 3); this lag

apparently resulted from a dephosphorylation that did not affect glucose 6-phosphate-independent glycogen synthase activity. If this explanation is correct, the same dephosphorylation should increase the apparent affinity of the enzyme for glucose 6-phosphate, since this parameter is a progressive function of phosphorylation state (Roach *et al.*, 1976; Brown *et al.*, 1977). Fig. 5 demonstrates the temporal relationship between phosphate release, glucose 6-phosphate-independent enzyme activity and activity expressed in the presence of 0.2 mM-glucose 6-phosphate. Adipose-tissue extracts from starved rats catalysed release of the first mol of phosphate per subunit of synthase without decreasing the dependence of enzyme activity upon glucose 6-phosphate (Fig. 5a). However, activity expressed in the presence of 0.2 mM-glucose 6-phosphate increased without delay, indicating that phosphate release decreased the apparent activation constant of the enzyme for glucose 6-phosphate.

Discussion

One of the original aims of the present study was to determine whether an impairment in adipose-tissue glycogen synthase phosphatase explained the marked inhibition of glycogen synthesis during

Fig. 4. Adipose-tissue glycogen synthase phosphatase activities in extracts from fed (●) and starved (○) rats at two substrate concentrations

Glycogen synthase was phosphorylated to 3.2 mol of phosphate per subunit with $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ and a combination of cyclic AMP-dependent and -independent protein kinases. The Sephadex G-25 column eluate containing labelled glycogen synthase was added to adipose-tissue extracts without further dilution for a final concentration of $50\text{ }\mu\text{g/ml}$ (a) or after a 10-fold dilution, i.e. glycogen synthase = $5\text{ }\mu\text{g/ml}$ (b). Protein-bound phosphate was determined at various times and the amount (pmol) of phosphate released at each time was calculated and is expressed per mg of adipose-tissue protein. The adipose-tissue extract prepared from starved rats was diluted so that the protein concentration in the incubation was approximately the same as that of extracts from fed rats (0.70 and 0.77 mg/ml for extracts from fed and starved rats respectively).

starvation. To examine phosphatase activity independently of changes in endogenous substrate(s) during starvation it was necessary to use exogenous substrate. Some of our conclusions must therefore be tempered by possible differences between the exogenous substrates tested and the endogenous adipose-tissue glycogen synthase.

With this limitation in mind, however, it is clear that adipose-tissue extracts from starved rats are not grossly deficient in glycogen synthase phosphatase activity. Both of the phosphorylated exogenous substrates tested were dephosphorylated at rela-

Fig. 5. Phosphate release and glycogen synthase activity assayed in the absence of and presence of 0.2 mM-glucose 6-phosphate

Glycogen synthase phosphorylated with a combination of cyclic AMP-dependent and -independent protein kinases was used as substrate. Glycogen synthase was assayed at the times indicated in the presence of zero (Δ , $\blacktriangle$), 0.2 mM ($\square$, $\blacksquare$) or 7.5 mM (constant, 51 munits/ml, not shown) glucose 6-phosphate. Activity is expressed as munits/ml of incubation mixture. Protein-bound phosphate ($\circ$, $\bullet$) was determined simultaneously. The adipose-tissue protein concentrations in the incubation were 0.70 and 1.27 mg/ml for extracts from fed and starved rats respectively. (a) starved rats; (b) fed rats.

tively similar rates by extracts from fed or starved rats. Low concentrations (approx. $5\text{ }\mu\text{g/ml}$) of exogenous muscle glycogen synthase were used in most experiments to magnify potential differences in substrate affinity of the phosphatases from fed and starved rats. We also determined glycogen synthase phosphatase activity using $50\text{ }\mu\text{g}$ of exogenous substrate/ml (Fig. 4). Phosphatase activity was 5-fold higher at this substrate concentration, suggesting that this concentration was approx. equal to the K_m (assuming Michaelis-Menten kinetics). Under these conditions, differences in V_{max} of the phosphatase from fed and starved rats should be apparent. No greater differences in phosphatase activity were seen at this higher substrate concentration.

It should be noted that the decrease in phosphatase activity in the starved animal would appear greater (i.e. up to a 60% decrease) if activity was expressed per mg of adipose tissue protein. This greater difference in phosphatase specific activity is a result of the increased protein concentration in extracts from starved rats. Whether or not this is a more valid way to express the data, the quantitative differences in phosphatase activity still appear insufficient to explain the marked differences in glycogen synthase activation in the fed and starved rat.

If the phosphatase activities in adipose tissue from fed and starved rats had different specificities for the phosphorylated sites on glycogen synthase, differential activation of glycogen synthase could result. However, the relationship between the extent of dephosphorylation and glycogen synthase activity (at 0 or 0.2 mM-glucose 6-phosphate) of exogenous substrate was the same whether dephosphorylation was catalysed by extracts from fed or starved rats (see Figs. 2, 3 and 5). Thus no preferential activation by extracts from fed rats was demonstrable.

There was, however, a striking difference in the effect of dephosphorylation on the glucose 6-phosphate-independent activity of the two exogenous enzyme substrates. Both phosphorylated substrates were largely glucose 6-phosphate-dependent (less than 10% activity in the absence of glucose 6-phosphate). The glucose 6-phosphate-independent enzyme activity of glycogen synthase phosphorylated by the cyclic AMP-dependent protein kinase increased as a function of dephosphorylation (Fig. 2). In contrast, glycogen synthase phosphorylated with both kinases (3 mol of phosphate/subunit) expressed only 20% activity in the absence of glucose 6-phosphate, even after adipose-tissue phosphatase catalysed the release of 2 mol of phosphate per subunit (Fig. 3). In addition, a delay preceded activation of glycogen synthase phosphorylated with both kinases (Figs. 3 and 5), but not that of glycogen synthase phosphorylated with the cyclic AMP-dependent protein kinase alone (Fig. 2).

Other workers have noted and extensively examined similar lag periods that precede activation of liver and adipose-tissue glycogen synthase (Gold & Segal, 1967; Stalmans *et al.*, 1971, 1974). Prior inactivation of phosphorylase appears to be prerequisite to the activation of glycogen synthase in the liver (Stalmans *et al.*, 1971, 1974), whereas in adipose tissue other, undetermined, factors are apparently responsible for the delay (Barash *et al.*, 1977). Our experiments with exogenous phosphorylated glycogen synthase demonstrate no lag in phosphatase activity measured by the release of $^{32}\text{P}_i$, or by increases in glycogen synthase activity assayed at a glucose 6-phosphate concentration of

0.2 mM (Figs. 3 and 5). Thus, at least with exogenous muscle glycogen synthase as substrate, the lag in enzyme activation does not reflect a latency in phosphatase activity, but can be explained by the non-linear relationship between the phosphorylation state of glycogen synthase and the glucose 6-phosphate-independent enzyme activity (Soderling *et al.*, 1977; Brown *et al.*, 1977). The observation that the activity of glycogen synthase determined with 0.2 mM-glucose 6-phosphate increased as a more direct function of dephosphorylation is consistent with the progressive relationship between the glucose 6-phosphate activation constant and the phosphorylation state of glycogen synthase (Roach *et al.*, 1976; Nimmo *et al.*, 1976; Brown *et al.*, 1977).

The phosphorylation state of the endogenous adipose-tissue glycogen synthase(s) is not known. However, there are differences in the kinetic properties of glycogen synthase in adipose tissue of fed and starved rats that suggest that the enzyme from starved rats may be more highly phosphorylated than that of fed rats (Eichner, 1976; Kaslow *et al.*, 1979). The high glucose 6-phosphate activation constant that characterizes the enzyme from starved rats is comparable with that seen when skeletal-muscle glycogen synthase is phosphorylated with both cyclic AMP-dependent and -independent protein kinases (Brown *et al.*, 1977). If the glucose 6-phosphate activation patterns shown in Fig. 1 for endogenous glycogen synthase are compared with those found with exogenous substrate, distinct similarities are seen. The endogenous enzyme from starved rats was activated only slowly, and after a lag period, a pattern seen clearly with highly phosphorylated exogenous substrate (Figs. 3 and 5). In contrast, the endogenous enzyme from fed rats, like the less highly phosphorylated exogenous substrate (Fig. 2), was rapidly activated and without delay. Thus differences in the phosphorylation states of glycogen synthase from fed and starved rats could account for the apparent divergent patterns of enzyme activation as measured by changes in glucose 6-phosphate-independent activity of glycogen synthase.

It is not surprising, in view of present knowledge, that partial dephosphorylation of glycogen synthase can have little effect on glucose 6-phosphate-independent enzyme activity. Yet the corollary, that changes in glucose 6-phosphate-independent activity do not accurately reflect phosphatase activity, has received little attention. We have shown here that marked differences in glycogen synthase activation profiles are accompanied by only modest decreases in glycogen synthase phosphatase activity. We have also demonstrated that one could explain the markedly reduced activation of glycogen synthase from starved rats by analogy to the activation profile seen with a highly phosphorylated exogenous

substrate. The endogenous glycogen synthase from starved rats is characterized by an increased activation constant for glucose 6-phosphate (Eichner, 1976). Whether this kinetic parameter correlates with increased phosphorylation, as is the case for skeletal-muscle glycogen synthase (Roach *et al.*, 1976; Nimmo *et al.*, 1976; Brown *et al.*, 1977; Cohen *et al.*, 1978), remains to be determined. Nonetheless the results presented here illustrate that decreases in glycogen synthase activation cannot be simply equated with decreases in phosphatase activity, but must be analysed in light of the phosphorylation state of the endogenous substrate, which is itself a primary determinant of the observed activation profile.

This work was supported by a grant from the National Heart, Lung and Blood Institute (HLB 12373). We would like to thank Dr. Harvey Kaslow for valuable discussion.

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