REVIEW LETTER

THE CONTROL OF GLYCOGEN METABOLISM IN THE LIVER

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1. Introduction

The glycogen stored in the liver is used as a reserve of glucose for the blood at the benefit of other tissues, mostly brain, erythrocytes and muscle; the hepatocyte itself uses only a small part of its carbohydrate for its own metabolic needs. It is therefore not surprising that, in the liver, the metabolism of glycogen is tightly regulated by the level of glycemia and by several hormones according to the general demand. In muscle, on the contrary, glycogen is a glycolytic fuel for the supply of ATP in anaerobic conditions and its metabolism is much more under the control of local factors.

In this review we shall discuss the enzymic mechanisms by which the metabolism of glycogen in the liver is controlled by glucose, glucocorticoids, glucagon and by glycogen itself. Insulin seems also to play a role, but its mode of action so far remains mysterious. A brief comparison between the regulation of glycogen metabolism in liver and muscle will be drawn.

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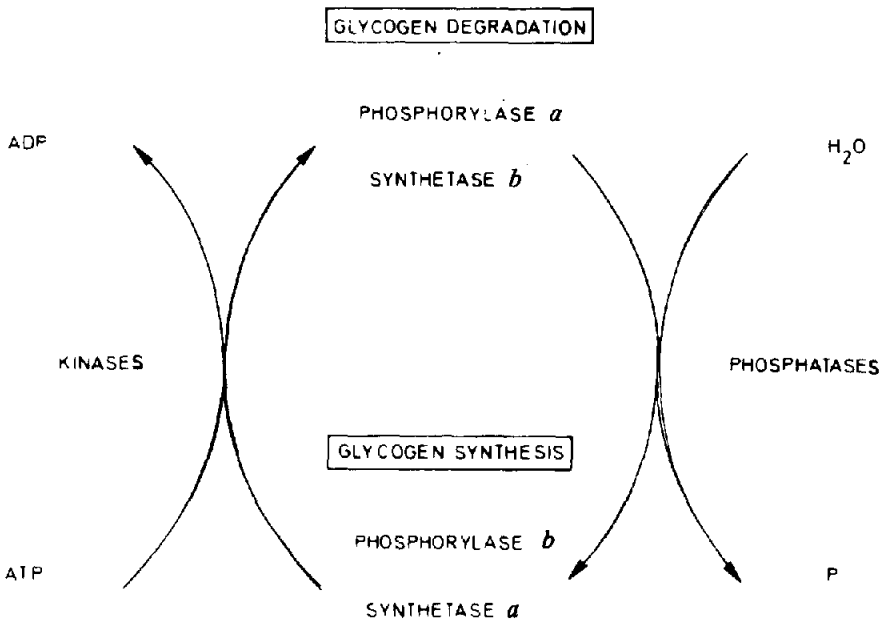

Fig. 1. Interactions of the enzymes controlling glycogen metabolism in the liver.

2. The rate limiting enzymes; their phosphorylation and dephosphorylation

As will be shown in section 3, the rate of glycogen synthesis in the liver is parallel to the activity of glycogen synthetase. There is also good evidence that glycogen degradation is entirely controlled by the activity of phosphorylase. This review will therefore be restricted to the control of these two rate limiting enzymes. Each of them exists in two forms, that are interconvertible by phosphorylation and dephosphorylation as schematically represented in fig. 1. The liver contains a phosphorylase phosphatase [1] and a phosphorylase kinase [2]; the latter enzyme is more active in the presence of cyclic AMP (see section 6). The liver phosphophosphorylase (phosphorylase *a*) is the active form of the enzyme; its activity is increased by 15 to 40% upon the addition of 10^{-3} M AMP [3,4]. The dephosphoenzyme (phosphorylase *b*) is completely inactive in the absence of AMP; its activity in the presence of the nucleotide is nearly zero in pig or rabbit liver [5], but reaches 15% of phosphorylase *a* in dog liver [3] and 25% in mouse liver [6].

The inactivation of glycogen synthetase in the presence of ATP in a muscle extract and the stimulation of this reaction by cyclic AMP was described in 1961 by Belocopitow [7]. The conclusive evidence that the muscle enzyme exists in two forms interconvertible by phosphorylation and dephosphorylation was later obtained by Friedman and Larner [8]. It is highly probable, although not definitively proven, that the same system occurs in the liver [9–11]. The main difference between phosphorylase and synthetase is that the active forms are the phosphophosphorylase and the dephosphosynthetase.

Liver phosphosynthetase requires glucose-6-phosphate for its activity; it is completely inactive in the presence of a physiological concentration of inorganic phosphate [12] whereas the dephosphoenzyme is nearly fully active in the same conditions [13]. One form is therefore inactive and the other active *in vivo*, whatever the concentration of glucose-6-phosphate. Other properties of the two forms have been described [12–16]. The phospho- and the dephosphosynthetase have been called respectively D (glucose-6-phosphate dependent) and I (glucose-6-phosphate independent) by Hizukuri and Larner [14], *b* (inactive) and *a* (active) by Mersmann and Segal [13]. The D and I designation is misleading because it gives the false impression that glucose-6-phosphate might be of importance in the *in vivo* activity of glycogen synthetase. We shall use only the *a* and *b* nomenclature, which conforms to that initially introduced by Cori and Green [17] for the 2 forms of muscle phosphorylase.

3. The conversion of the rate limiting enzymes *in vivo*

3.1. *The effect of glucose and glycogen*

There is ample experimental evidence that glycogen synthesis and degradation are controlled by the concentration of glucose in the blood. A deposition of 10 mg polysaccharide/hr/g liver has been observed after fasting and refeeding in adrenalectomized [18,

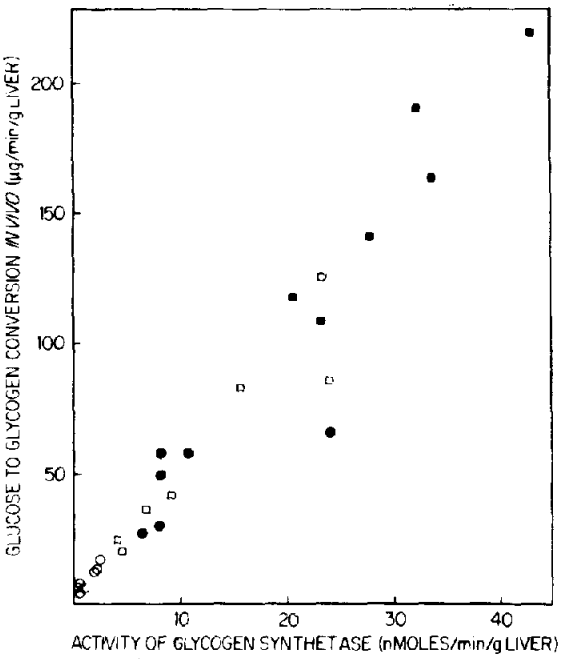

Fig. 2. Correlation between the activity of liver glycogen synthetase *a* measured at 0° and the rate of the *in vivo* conversion of glucose to liver glycogen in the same animal. The *in vivo* conversion was measured by the incorporation of 6-^{3}H-glucose in 1 min; (○) control mice; (●) mice injected with glucose (1 mg/g, i.v. 5 min before the experiment); (□) prednisolone treated mice (1 mg/20 g, s.c. 3 hr before); (■) prednisolone treated mice injected with glucose. Correlation coefficient 0.94 ($p < 0.001$). From De Wulf and Hers [28].

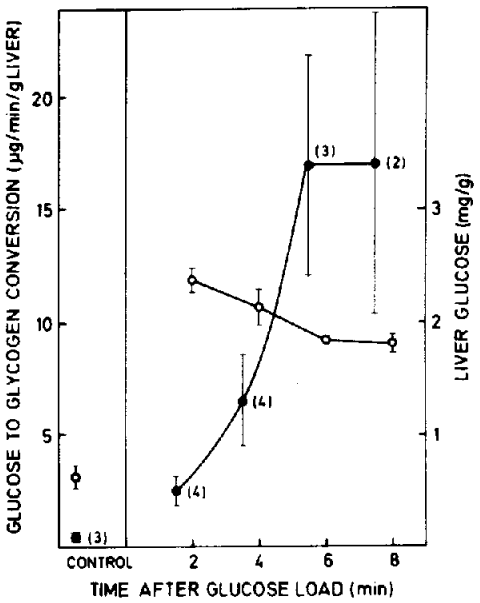

Fig. 3. Change in the rate of glycogen synthesis after a single glucose load. Glucose was injected intravenously into mice fasted for 24 hr in a single dose of 1 mg/g body weight. At various intervals afterwards, a trace amount of U-^{14}C-glucose was administered intravenously and the animals were killed 1 min later. (○) glucose concentration in the liver at the end of the experiment; (●) amount of glucose converted into glycogen in 1 min. Vertical bars represent ± the standard error of the means; the number of experiments is given in parentheses. From De Wulf and Hers [22].

19] and in diabetic rats [20, 21] as well as in normal animals.

It is now firmly established [22] that the increase in the rate of glycogen synthesis due to glucose is not the result of a push given along the metabolic pathway leading to glycogen. The glucose effect is indeed much greater than the change in glycemia and is concomitant with a decrease in the intrahepatic concentration of the intermediary metabolites: UDPG and glucose-6-phosphate. The decisive effect of the administration of glucose is the conversion of glycogen synthetase into its active form. The *in vivo* rate of glycogen synthesis is indeed highly correlated with the amount of synthetase *a* in the liver (fig. 2).

When a glucose load is given intravenously, the rate of glycogen synthesis starts to increase only after a short latency (see fig. 3), reaches its maximum after 5 to 6 min and then decreases progressively to come back to the initial value soon after the glycemia has been normalized [22]. The latency indicates that the effect of glucose on the activity of synthetase is indirect. As this effect has been obtained in animals made acutely diabetic by the administration of anti-insulin serum [23], it seems not to be mediated by insulin. In agreement with this conclusion, the activation of glycogen synthetase by a glucose load has been recently observed in perfused rat liver [24, 25].

An increase in the concentration of glucose *in vivo* [26–28] or in the isolated perfused liver [24, 25] initiates a slight decrease in the amount of active phosphorylase; this effect is much less striking than that on glycogen synthetase. It is a usual finding that the activity of phosphorylase *a* measured in a fresh liver homogenate largely exceeds what would be expected from the actual rate of glycogenolysis. This may be due to a very rapid activation of the enzyme in the process of homogenization or to some other reasons. In the dog only, very low values of liver phosphorylase have been observed after the administration of glucose and insulin [29].

The effect of glycogen concentration on the rate of glycogen synthesis has, to our knowledge, not been systematically investigated. In the muscle, there is an inverse relationship between the glycogen content of the tissue and the percentage of synthetase in the active form [30] and this has been explained by an inhibition of glycogen synthetase phosphatase by glycogen [31]. Although the liver can accumulate glycogen to a much higher concentration than the muscle, it appears probable that there exists in this tissue also a feedback mechanism that prevents the excessive deposition of the polysaccharide.

3.2. *The effect of insulin*

It has been observed repeatedly that insulin, when given together with glucose, causes a glycogen deposition in the liver [32, 33] and, more recently, this effect has been attributed to an activation of glycogen synthetase [29]. As glucose given alone is also very efficient, it is not easy to dissociate what part of the effect is due to insulin itself. It seems, however, that, with insulin, the effect of a glucose load is obtained at a lower level of glycemia [22].

Insulin alone does not induce an activation of glycogen synthetase in the liver of normal animals,

at least within a few minutes [6]. It does, however, activate the enzyme within 5 to 15 min in diabetic rats, if administered only two days after the alloxan treatment [34, 35], and this effect is not related to a drop in the concentration of cyclic AMP in the liver [34]. An activation can also be obtained in rats that have been diabetic for a longer time, but then it requires about one hour [36].

3.3. *The effect of glucocorticoids*

The stimulation of liver glycogen synthesis by glucocorticoids was described in 1940 by Long et al. [37]; it was observed both in normally fed and in fasted animals and is therefore not secondary to the stimulation of gluconeogenesis. Accordingly, glucocorticoids increase the rate of conversion of blood glucose into liver glycogen [38, 39]. The effect has also been obtained in diabetic animals [40, 41] and is therefore not insulin-dependent; contradictory results [34, 42] have been obtained only in experiments which were of too short duration (2 hr).

The glucocorticoids require approximately 3 hr to stimulate glycogen synthesis. At that time, the concentration of glucose-6-phosphate and UDPG are markedly reduced in the cell and the synthetase is converted into its active form [43, 44]. Here also, there is a striking parallelism between the activity of glycogen synthetase and the actual rate of glycogen synthesis (see fig. 2). It is also shown in the same figure that the administration of glucose to mice treated with prednisolone 35 hr previously, further increases the activity of glycogen synthetase and the rate of glycogen synthesis.

After the administration of corticoids, there is a decrease in the activity of glycogen phosphorylase [28]. This decrease is however much less important than the activation of glycogen synthetase

3.4. *The effect of glucagon*

Sutherland and Cori [45] were the first to observe that epinephrine and glucagon cause the activation of phosphorylase in liver slices. Due to the high basal activity of phosphorylase *a* (see section 3.1.) the changes in phosphorylase activity induced by the hormone *in vivo* or in a perfused liver, are proportionally much smaller than the changes in glucose production. In the experiment taken from Weintraub et al. [46] and reported in fig. 4, there is a two fold increase in enzymic activity but a 10 to 20 fold increase in glucose production.

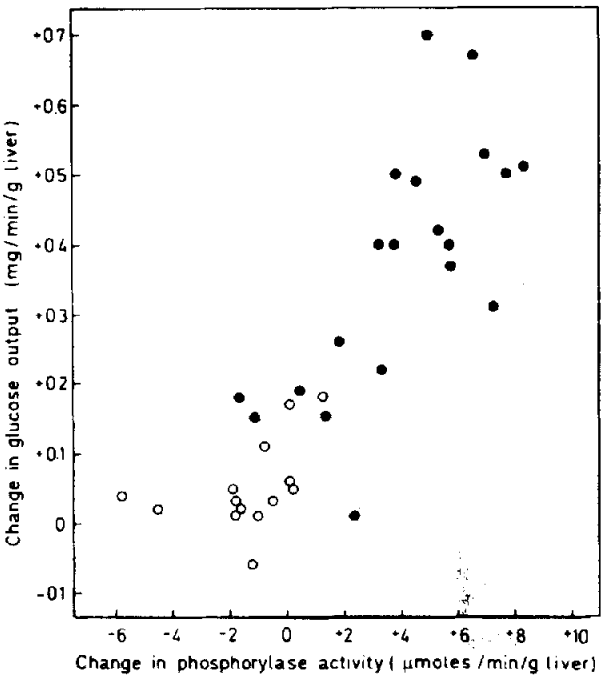

Fig. 4. Correlation between the changes in the activity of liver glycogen phosphorylase and in glucose output in perfused rat liver. For each liver, the mean change in glucose output is plotted as a function of the mean change in phosphorylase activity. For the control livers, base line phosphorylase activity averaged 11 U/g and glucose output 0.025 mg/min/g liver. (○) control livers; (●) livers perfused with glucagon (from 0.01 to 5 μg). Correlation coefficient: 0.84 ($p < 0.001$). This figure was drawn with the data in tables 1 and 2 in Weintraub et al. [46].

One observes, however, a good correlation between the two effects. *In vivo*, the increase in the activity of phosphorylase is also about 2 fold [47] or much less [27, 28]. The effect of glucagon on glycogen synthetase is more apparent as a nearly complete inactivaction of the enzyme can be obtained [28, 29]. The hormone cancels the activation of glycogen synthetase by glucose and also reduces considerably the activity of the enzyme that has been activated by glucocorticoids. Cyclic AMP or epinephrine have a similar effect when given intravenously. This inactivation is obtained in 1 to 3 min [28]. At physiological concentration, glucagon is much more glycogenolytic than is epinephrine [48].

The increase in the concentration of cyclic AMP in the liver after the administration of a small dose

(0.5 ng/g) of glucagon to mice precedes the inactivation of glycogen synthetase by 1–2 min [49]. This sequence of events is in agreement with the role of the nucleotide as second messenger in the hormonal action.

4. The control of phosphorylase phosphatase by glucose, glycogen or glucocorticoids

The regulation of phosphorylase phosphatase is most conveniently studied in a liver extract that has been filtered through a column of Sephadex G-25 [50]. In this preparation, obtained from fed mice, the conversion of phosphorylase *a* into *b* at 20° is complete in approximately 20 min. This is schematically represented by the descending full line in fig. 5. The conversion is accelerated (dotted line a, in fig. 5) by glucose, caffeine or nicotinamide added to the preparation, and retarded (dotted line r) by glycogen. The stimulation of liver phosphorylase phosphatase by glucose is apparently specific for this hexose as it was not obtained with any other sugar or sugar derivative that was asayed. It is concentration dependent with an apparent K_m equal to 0.13 g/100 ml (7 mM) glucose and is additive to the effect of caffeine.

When the liver is obtained from mice that have been treated with prednisolone a few hours previously, the activity of phosphorylase phosphatase is 3 to 10 times higher than normal (dotted line a) and can be further stimulated by glucose or caffeine (dotted line a'). As the action of glucocorticoids on glycogen synthesis requires several hours to be complete and as the properties of phosphorylase phosphatase appear not to be modified by the treatment, it is very probable that the steroids initiate a more rapid synthesis of the enzyme.

The observations reported in this paragraph give an appropriate explanation for the lower activity of phosphorylase in the liver *in vivo* after a treatment of the animals with glucose [26–28] or with glucocorticoids [28].

5. The control of synthetase phosphatase by phosphorylase

In a fresh liver extract, glycogen synthetase is mostly in the *b* form and can be converted into *a* by incubation

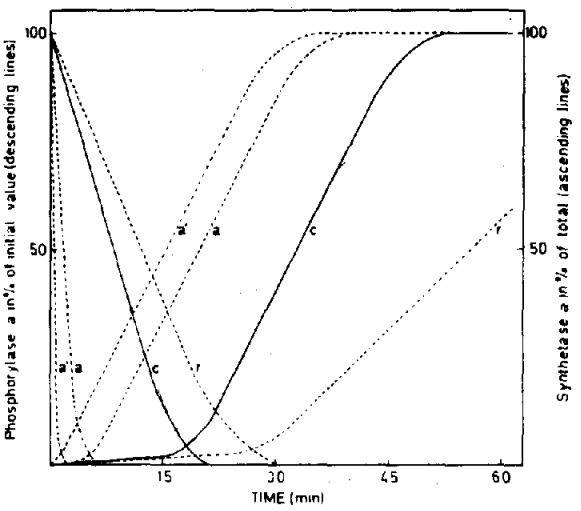

Fig. 5. Schematic representation of the inactivation of phosphorylase (descending lines) and of the activation of glycogen synthetase (ascending lines) as observed in a liver Sephadex filtrate incubated at 20°. c) control filtrate; a) control filtrate incubated in the presence of 0.5% glucose or filtrate from mice treated with prednisolone; a') filtrate from mice treated with prednisolone incubated in the presence of 0.5% glucose; r) control filtrate incubated in the presence of 6% glycogen. The original data from which the scheme is constructed can be found in [50, 52, 53].

at 20°; this conversion is, however, preceded by a latency period of approximately 20 min [51]. The activation pattern is best evidenced when all the synthetase has been first converted into the *b* form by an injection of glucagon shortly before sacrifice and when the liver extract has been filtered through Sephadex G-25 [52]. The activation then occurs as schematically shown in fig. 5 by the ascending curve c (full line). With this preparation, it has been observed that all effectors (glucose, caffeine, nicotinamide and glucocorticoids) that increase the activity of phosphorylase phosphatase, also shorten the latency period in the activation of glycogen synthetase (ascending lines a and a'); glycogen has the reverse effect (ascending line r). In all conditions the latency period corresponds to the time required to inactivate phosphorylase. The activity of synthetase phosphatase itself (slope of the ascending curve) is not modified by the effectors, except by glycogen which causes a slight inhibition.

These facts were explained when it was shown [53]

that phosphorylase *a* is a strong inhibitor of liver synthetase phosphatase: when added before the end of the latency, it delays the activation of glycogen synthetase; when added later, it stops the reaction, causing the appearance of a new latency. Phosphorylase *b* is much less inhibitory. The inhibition of synthetase phosphatase by phosphorylase *a* is cancelled by AMP; the activation of glycogen synthetase proceeds then without latency and at a rapid rate. The latency is also suppressed when phosphorylase is combined with specific antibodies. It is clear therefore that the latency is entirely explained by the inhibitory action of phosphorylase *a*. The previous suggestion [52, 54] that synthetase phosphatase might exist in two interconvertible forms has thus been disproved.

The activation *in vivo* of glycogen synthetase by glucose or glucocorticoids is therefore also explained by the increased activity of phosphorylase phosphatase; once phosphorylase is inactivated, synthetase phosphatase, now released from inhibition, can activate glycogen synthetase.

Synthetase phosphatase requires the presence of a small amount of glycogen to be active *in vitro*. It is poorly active in preparations obtained from fasted animals, but recovers its full activity if 0.5% glycogen is added [6, 55]. The enzyme is also inactive in liver extracts [56] and in perfused livers [25] obtained from fasted adrenalectomized rats, despite the fact that glycogen synthesis rapidly occurs *in vivo* upon refeeding of these animals [18, 19].

The activation of glycogen synthetase proceeds at a normal rate in a liver filtrate obtained from alloxan diabetic mice [6]. Synthetase phosphatase has however been reported to have lost a part of its activity in the liver of diabetic animals [35, 54]. It is not clear if the latter effect can be explained by a lack of glycogen or by an excess of phosphorylase *a* in the preparation. *In vivo*, fasted diabetic rats synthetize glycogen upon refeeding at an essentially normal rate [20, 21].

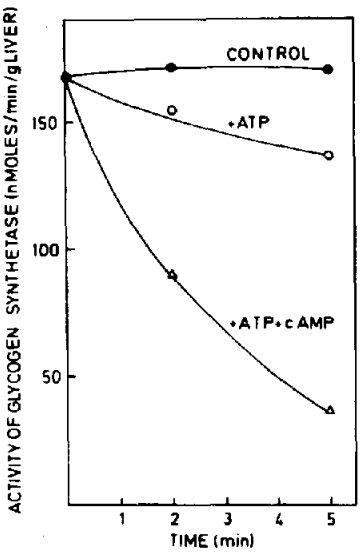

Fig. 6. The influence of ATP and cyclic AMP on the inactivation of glycogen synthetase. A liver extract was obtained from mice injected with glucose (2 mg/g) 6 min before death. This extract, at a final concentration of 12.5%, was incubated at 20° in 100 mM NaF, 5 mM caffeine and 5% glycogen either without further addition (•) or in the presence of 2.5 mM ATP and 2.5 mM Mg acetate (○) or 2.5 mM ATP, 2.5 mM Mg acetate and 10 μM cyclic AMP (△). From De Wulf and Hers [9].

6. The control of the kinases by cyclic AMP

In a liver extract, phosphorylase kinase is stimulated by cyclic AMP; a half maximal effect is obtained with 2×10^{-7} M nucleotide [57]. The muscle enzyme has been extensively purified, and, in that case, it has been shown that phosphorylase kinase itself exists as an activated and non-activated form [58]; the activation occurs by phosphorylation through a phosphorylase kinase kinase that is cyclic AMP dependent [59]. This kinase kinase acts also on other proteins [60], including the dephosphosynthetase [61, 62] and has been called a "protein kinase" [60]. It is probable, although not demonstrated, that the same cascade of reactions occurs in the liver [63].

In a liver extract, glycogen synthetase kinase is stimulated by cyclic AMP (see fig. 6) and has a K_m that is also close to 2×10^{-7} M [9, 64]. A partially purified kinase displays, however, a slightly higher affinity [10]. The muscle protein kinase inactivates liver synthetase *a* [11].

7. A comparison between liver and muscle

A detailed account of the regulation of glycogen metabolism in the muscle exceeds the scope of this review. It is however of interest to underline the differences that exist between the liver and muscle systems.

Muscle glycogen synthetase D (*b*) is less inhibited by inorganic phosphate than the liver *b* enzyme [65, 66]. The concentration of glucose-6-phosphate increases markedly in muscle during tetanic contraction and may be sufficient to allow the D form to be active [67]. The regulation of glycogen synthesis in the muscle operates therefore not only by interconversion of the active and inactive forms of the synthetase but also by a change in the concentration of glucose-6-phosphate [67].

As there is no free glucose in muscle [68], a control of glycogen metabolism by the level of glycemia is not possible. Phosphorylase phosphatase is, however, slightly stimulated by glucose [69] and this effect has recently been shown to be dependent upon the presence of AMP [70]. Another important difference between the two tissues is that muscle synthetase phosphatase is not inhibited by phosphorylase [71]. Finally, it seems that in muscle the main stimulator of phosphorylase kinase is Ca^{2+} [72], which is released at the onset of muscular contraction.

8. Conclusion

8.1. *The overall mechanism*

We summarize in fig. 7 the biochemical mechanisms that regulate glycogen metabolism in the liver. The level of active phosphorylase and of active synthetase is controlled by the antagonistic effects of the kinases and phosphatases. It has been shown that, at least in the muscle, the same protein kinase acts as a phosphorylase kinase kinase and as a synthetase kinase. This enzyme is cyclic AMP-dependent and its action causes the arrest of glycogen synthesis together with the onset of glycogenolysis. Phosphorylase phosphatase is stimulated by glucose, inhibited by glycogen, and its total amount is augmented by glucocorticoids. Being controlled both on the side of its kinase and of its phosphatase, phosphorylase is the most highly regulated enzyme in the system. By its action on synthetase phosphatase, phosphorylase acts as a second messenger in the control of glycogen synthesis by glucose and by glucocorticoids. These latter agents are antagonistic to cyclic AMP: they cause the arrest of glycogenolysis, followed by the onset of glycogen synthesis.

When protein kinase is stimulated by cyclic AMP, synthetase phosphatase is inactive, due to the inhibi-

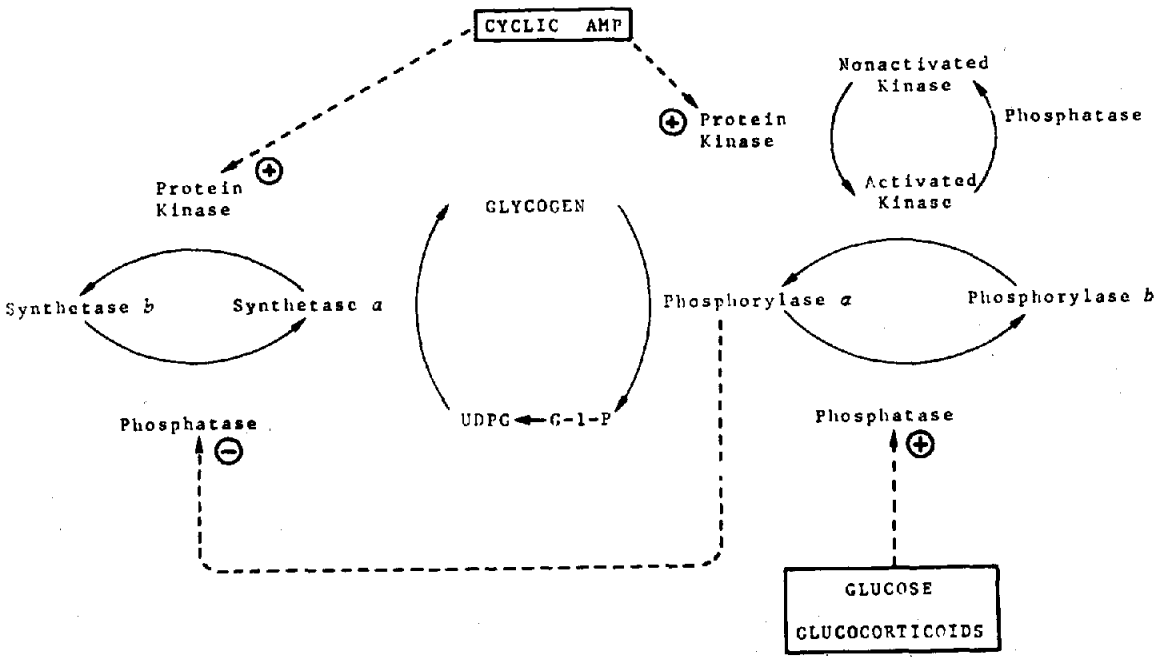

Fig. 7. The regulation of glycogen metabolism in the liver [53].

tion by phosphorylase *a*; there is therefore no futile recycling of the two forms of glycogen synthetase when the level of cyclic AMP is elevated. It appears very probable however that, in other circumstances, the kinases and the phosphatases operate simultaneously, and that the actual level of active phosphorylase or of active synthetase is the result of a balance between phosphorylation and dephosphorylation. If this was not the case, phosphorylase and synthetase would always be 100% active or 100% inactive. Yet, we know that phosphorylase is not necessarily completely in the *a* form when the level of cyclic AMP is elevated: this indicates that the stimulated kinase is kept in check by the phosphatase, stimulated itself by a higher glucose level.

At normal levels of glycemia, the synthetase is almost entirely in the inactive form and can be activated by glucose within a few minutes or by glucocorticoids within a few hours. This implies that some of the phosphorylase was in the active form. Under the action of glucose or of glucocorticoids, the inactivation of phosphorylase is presumably almost complete, otherwise synthetase phosphatase would not operate. As the two treatments have additive effects, it appears that each of them separately is unable to increase phosphatase activity up to the point where it completely overcomes the kinase. Under the same conditions, the activation of synthetase is also incomplete (see fig. 2), indicating that synthetase phosphatase has to compete with synthetase kinase.

8.2. *Regulation by glucose*

The abundance of glucose in the body governs the flow of many metabolites and more particularly controls the carbohydrate store in the liver. This has now been explained by an apparently direct control of phosphorylase phosphatase by glucose. This effect is shared by no other sugar and explains that glycogen degradation is stopped when the concentration of glucose is elevated and *vice versa*. By accelerating the conversion of phosphorylase *a* into *b*, a high glucose level allows the activation of glycogen synthetase. This indirect effect explains the latency that precedes the activation of glycogen synthetase *in vivo* as well as *in vitro*.

The apparent K_m of phosphorylase phosphatase for glucose in a liver Sephadex filtrate is of the order of 0.13 g/100 ml. If the same value applies *in vivo*, the enzyme would be about 50% active at normal glucose concentration (0.15 g/100 ml in fed mice). Under this condition, however, synthetase is still mostly in the inactive form, indicating that phosphorylase *a* is present. This situation can be understood if the activity of phosphorylase phosphatase at normal level of glycemia is balanced by the basal activity of phosphorylase kinase; only an excess of glucose will allow the phosphatase to overcome the kinase.

8.3. *Regulation by insulin*

We do not know, at the present time, how insulin might interfere with one of the regulatory mechanisms shown in fig. 7.

8.4. *Regulation by glucocorticoids*

Glucocorticoids allow the synthesis of glycogen to proceed in the fasting state, i.e. at a low glucose level, and they bring the concentration of polysaccharide up to very high values. These effects are easily explained by the large increase in phosphorylase phosphatase caused by treatment with these hormones. As shown by the dotted line a in fig. 5, this activity in the absence of glucose is now equal to that obtained normally under full stimulation by the hexose. Corticoid treatment has thus the same effect as protracted hyperglycemia. Furthermore, a higher concentration of glycogen will be required in order to reduce the activity of the excess of phosphorylase phosphatase to the critical point where eventually no more glycogen synthesis occurs.

8.5. *Regulation by glucagon*

Great emphasis has been put on the regulation of glycogen breakdown by glucagon, presumably because its mechanism has been clear for many years. It now appears that glucagon not only activates glycogen phosphorylase but also prevents glycogen synthesis by the double lock of a stimulation of synthetase kinase and of an inhibition of synthetase phosphatase by phosphorylase *a*. It appears too that this is only one part of the regulatory mechanism and that glucose concentration is also of great importance. It is not clear, at the present time, whether the glycogenolysis in the fasting state is initiated by the hypoglycemia itself or by a secondary secretion of glucagon.

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