Cellular and hormonal control in glycogen metabolism — recent advances

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IN ANIMAL TISSUES, an easily available source of energy is the polysaccharide glycogen. It occurs mainly in liver and muscle but is also present in lesser amounts in other tissues, such as the heart, kidney, brain, skin and blood cells. The glycogen molecule is composed of over 120,000 glucosyl units, linked together in such a way that it assumes a "tree-like" appearance. The glucose molecules are joined to one another by an alpha-1, 4-linkage and at the branch points by an alpha-1, 6-linkage. The structure of glycogen, as studied by chemical and enzymic methods, has been reviewed by Manners (1962). Recent studies, using purified enzymes, confirm the multiple branching in the glycogen molecule (Bathgate and Manners, 1966).

The molecular weight of glycogen has been shown to vary from 8,000 to 20 million, depending on the method of extraction, the nutritional state and pathological condition of the tissue from which the glycogen is extracted (French, 1964; Edstrom, 1970). Drochmans and Danten (1968) have postulated that the glycogen molecules aggregate to form large particles ("rosette form") called alpha-particles and these, under acidic conditions, break up into smaller beta-particles. In the cell, the glycogen particles are found usually in the cytoplasm and in the lysosomes. They may also be found in the mitochondria and in the nucleus in certain pathological conditions.

The Pathways of Glycogen Metabolism

The study of glycogen metabolism in man has been intensive in the last 30 years. It began with Cori, Schmidt and Cori (1939) who showed that glycogen can be synthesised *in vitro* from glucose-1-phosphate by the enzyme phosphorylase. The same enzyme was also shown to be responsible for its degradation. By the early fifties, the enzymic degradation pattern of glycogen had been worked out.

Studies of defects in glycogen metabolism and glycogen storage diseases led to further investigations into alternative pathways for glycogen breakdown and synthesis. Leloir and Cardini (1957) showed that glycogen can be synthesised in tissues

from uridine diphosphate glucose by an enzyme "glycogen synthetase". The study of glycogen storage disease, Type V (McArdle's disease), in which muscle phosphorylase is absent, showed accumulation of glycogen in the muscle (Schmid and Mahler, 1959).

Adrenaline, a hormone which activates the enzyme phosphorylase, increased glycogen breakdown but not its synthesis. Grillo and Ozone (1962) showed that when glycogen in foetal liver was detectable, only glycogen synthetase activity was present while phosphorylase activity was still undetectable at this stage. These findings clearly show that the main pathway for glycogen synthesis *in vivo* is via the action of glycogen synthetase, while phosphorylase remains as one of the enzymes responsible for its degradation. The importance of a non-phosphorolytic pathway for glycogen breakdown in tissues is seen in glycogen storage disease Type II (Pompe's disease) in which there is no lysosomal acid glucosidase present. In this disease, excessive amounts of glycogen accumulate in tissues even though phosphorylase activity is present (Hers, 1963).

Glycogen breakdown

The degradation of glycogen in mammalian tissues is catalysed by enzymes which can be classified as either phosphorolytic or non-phosphorolytic. The former consists of the enzyme glycogen phosphorylase which successively cleaves the alpha-1, 4-linkages in the outer chains of the glycogen molecule to produce glucose-1-phosphate (Morgan and Parmeggiani, 1964). Phosphorylases are found in tissues where glycogen is present. Immunologically distinct phosphorylases, which respond differently to metabolites and hormones, have been isolated from muscle and liver tissues. However, phosphorylase is not capable of hydrolysing an alpha-1, 6-linkage or by-passing it. This results in a phosphorylase limit dextrin (Walker and Whelan, 1960).

Some of the side chains at this stage will contain four glucose residues. A transferase enzyme (alpha-1, 4 alpha-1, 4 glucan transferase) transfers a trisaccharide unit from these side chains to another branch, leaving behind a single glucose unit attached by a -1, 6-linkage. Another enzyme, amylo-1, 6-glucosidase, hydrolyses the 1, 6-linkage to produce glucose and through this action exposes more alpha-1, 4-linked glucose residues to further action by the phosphorylase. Thus, phosphorylase, the transferase enzyme and the amylo-1, 6-glucosidase, acting together, can completely degrade glycogen. The glucose-1-phosphate formed is mainly metabolised by its conversion to glucose-6-phosphate (by the enzyme phosphoglucomutase) or through the uronic acid pathway.

Dietary glycogen is degraded in the digestive tract by non-phosphorolytic pathways (Rutter and and Brosemer, 1961). Alpha-amylase attacks glycogen by its random hydrolytic splitting of internal alpha-1, 4-linkages to produce oligosaccharides (mainly maltose and maltotriose) and alpha-limit dextrins, which contain the alpha-1, 6-linkages. Alpha-glucosidases act (possibly at neutral pH) on the oligosaccharides and alpha-limit dextrins to produce glucose. The glycogen present in lysosomes is degraded by an exo-enzyme called acid-alpha-glucosidase, which acts at acid pH, to form glucose. This acid-alpha-glucosidase is believed to be capable of hydrolysing both the 1, 4- and 1, 6-linkages of glycogen (Brown, Jeffrey and Brown, 1969).

Glycogen Synthesis

In mammalian tissues, the first step in the synthesis of glycogen from glucose is the phosphorylation of glucose to glucose-6-phosphate by either of the enzymes hexokinase or glucokinase, using ATP as the phosphate donor. The glucose-6-phosphate is an important metabolite. It could be degraded either via the glycolytic pathway or the direct oxidative pathway. Glucose-6-phosphate is on a regulation site of glycogen metabolism. It could be reconverted to free glucose by the enzyme glucose-6-phosphatase or it could be converted to glucose-1-phosphate by the enzyme phosphoglucomutase. The latter is a reversible reaction.

Glucose-1-phosphate also occupies a key position in glycogen metabolism. During glycogen synthesis, it reacts with uridine triphosphate to form uridine diphosphate glucose, the reaction being catalysed by the enzyme uridine diphosphate glucose pyrophosphorylase. The reaction is reversible and the enzyme activity is dependent on magnesium ions. This enzyme, which is found in all tissues where glycogen is synthesised, is inhibited by high concentrations of inorganic phosphate and uridine diphosphate.

In the presence of a "glycogen primer" and the enzyme uridine diphosphate glucose alpha-glucan tranglucosylase (glycogen synthetase), glucosyl residues are transferred from uridine diphosphate glucose to the non-reducing ends of the primer resulting in an elongation of the external chains. The additional glucosyl residues are linked to one another by alpha-1, 4-linkages. When the outer chains of the glycogen molecule have each been lengthened by about eight glucose units, a branching enzyme (amylo-1, 4 1, 6-transgluco-

sidase) acts to remove a segment of the alpha-1, 4-linked chain, consisting of about seven glucose residues, to another part of the glycogen molecule to form an alpha-1, 6-linkage, thus creating a branch point in the molecule. The sequential action of glycogen synthetase and the branching enzyme results in multiple branched "tree-type" glycogen molecule (Manners, 1968).

Activation of Enzymes involved in Glycogen Metabolism

Some of the enzymes involved in glycogen metabolism exist in two forms — an active form and an inactive form. They are interconvertible, either reversibly or irreversibly. These molecular interconversions act as a control mechanism in glycogen synthesis and breakdown. The glycogen synthetase and glycogen phosphorylase activities are influenced by intracellular and extracellular factors such as metabolites, hormones and ions. The activities of these two enzymes are thought to be primarily responsible for regulating net glycogen turnover.

Glycogen phosphorylase

Mammalian muscle glycogen phosphorylase exists in two forms. The more active phosphorylase *a* is a tetramer with a molecular weight of about 370,000 and is active in the absence of AMP. The less active phosphorylase *b* is a dimer with a molecular weight of about 185,000. It is activated by high concentrations of AMP but inhibited by high concentrations of ATP or glucose-6-phosphate. The phosphorylases *a* and *b* are readily interconvertible via a specific phosphatase and a specific kinase (Fischer and Krebs, 1966).

$$\text{phosphorylase } a + 4H_2O \xrightarrow[\text{phosphorylase } a \text{ phosphatase}]{} 2 \text{ phosphorylase b} + 4P$$

$$2 \text{ phosphorylase } b + 4ATP \xrightarrow[Mg^{++};\ \text{phosphorylase } b \text{ kinase}]{} \text{phosphorylase } a + 4 \text{ ADP}$$

Thus, increased phosphorylase activity can be brought about by either converting the phosphorylase *b*-form to the *a*-form or by activating the phosphorylase *b* by increasing the concentrations of AMP or decreasing the concentration of ATP or glucose-6- phosphate. The *a*-form is predominant in "activated" tissue, such as contracting muscle, and the *b*-form is found in "resting" tissue. The liver and muscle phosphorylases are similar but the liver enzyme is a dimer and no cleavage of the molecule occurs during the conversion of the 'a'-form to the 'b'-form. Adrenaline increases the conversion of phosphorylase *b* to *a* in liver and muscle and glucagon of that in liver only.

The phosphorylase *b* kinase itself exists in two forms. The inactive form can be converted to the active form by adrenaline or glucagon, in the presence of divalent ions and ATP. The two forms are interconvertible and the activation is brought about by either one of the following reactions (Krebs, et al. 1966; Drummond and Duncan, 1968).

$$\text{phospohrylase } b \text{ kinase} + ATP \text{ (inactive form)} \xrightarrow[Mg^{++}]{\text{phosphorylase } b \text{ kinase-kinase}} \text{phosphorylase } b \text{ kinase-phosphate (active form)}$$

$$\text{phosphorylase } b \text{ kinase} + ATP \text{ (inactive form)} \xrightarrow[Ca^{++}]{\text{Kinase activating factor (KAF)}} \text{phosphorylase } b \text{ kinase-phosphate (active form)}$$

It is believed that the former mechanism is under hormonal control while the latter is under neural control (Shimazu and Anakawa, 1968). The activation can also be brought about by limited digestion with trypsin. The inactivation of the phosphorylase *b* kinase has not been fully investigated.

Cyclic 3, 5-AMP plays an important role in glycogen metabolism, particularly in its activation of the kinases. It is produced from ATP by the action of the enzyme adenyl cyclase.

This enzyme is activated by adrenaline and glucagon (Fig. 1). The increase in glycogen phosphorylase activity is due to the increase in cyclic 3, 5-AMP concentrations in tissues (Sutherland and Robison, 1966).

$$\text{ATP} \xrightarrow{\text{adenyl cyclase}} \text{cyclic 3, 5-AMP} + \text{pyrophosphate}$$

Glycogen synthetase

Glycogen synthetase (UDPG-alpha-glucan transglucosylase) exists in two forms in tissues where glycogen synthesis takes place. They are interconvertible by phosphorylation and dephosphorylation as shown below. (Larner et al. 1968).

$$\text{Glycogen synthetase } a \text{ (I-form, active)} \xrightarrow[\text{ATP, Mg}^{++}]{\text{Synthetase Kinase}} \text{Glycogen synthetase } b \text{ (D-form, inactive)}$$

$$\text{Glycogen synthetase } b \text{ (D-form, inactive)} \xrightarrow[\text{H2O}]{\text{Synthetase phosphatase}} \text{Glycogen synthetase } a \text{ (I-form, active)}$$

The D-form of the enzyme in liver (phosphosynthetase) requires glucose-6-phosphate for its activity while the I-form (dephosphosynthetase) is independent of glucose-6-phosphate for its activity. The D-form is completely inhibited by physiological concentrations of inorganic phosphate, whereas the I-form is nearly fully active under the same conditions (Mersmann and Segal 1967). One form is therefore active and the other inactive *in vivo* whatever the concentration of glucose-6-phosphate.

The conversion of the I-form (active) to the D-form (inactive) can also be brought about by either a kinase activating factor (KAF) which is a calcium-activated proteolytic enzyme or by a proteolytic enzyme like trypsin (Appleman et al. 1964). In the latter two cases, the D-form of the enzyme cannot be reconverted to the I-form. Adrenaline and glucagon, which increase the cyclic 3,5-AMP concentration in tissues, activate the glycogen synthetase kinase. Other cyclic 3,5-nucleotides also have the same effect (Wallas et al. 1968).

In normal tissues, the glycogen synthetase exists predominantly as the inactive or *b*-form and only a small amount of active or *a*-form is present (De Wulf et al. 1968). In hyperglycemia the *b*-form is converted to the *a*-form (Buschiazzo et al. 1970). The *b*-form of the enzyme has a low affinity for UDPG and glucose-6-phosphate and is only active in the presence of high concentrations of glucose-6-phosphate. The *a*-form, on the other hand, has

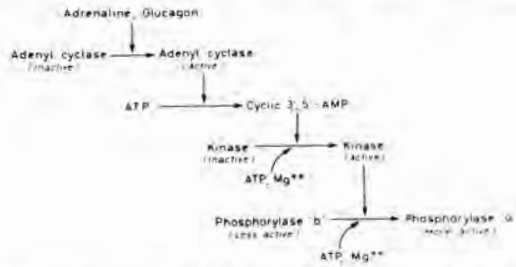

Fig 1 THE STEPS INVOLVED IN THE ACTIVATION OF PHOSPHORYLASE BY ADRENALINE OR GLUCAGON

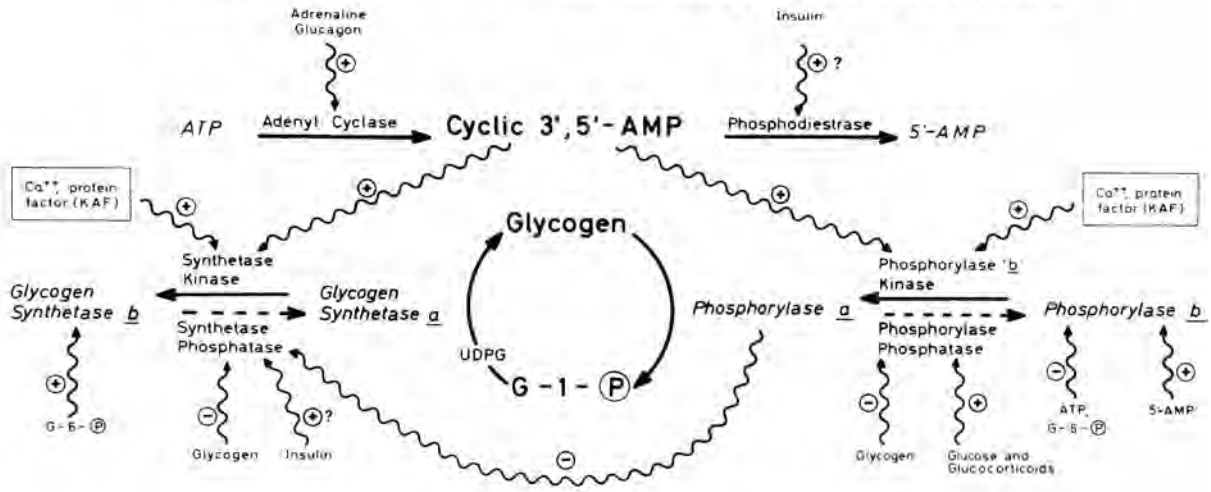

Fig 2 ***Influence of cyclic AMP and other regulators on glycogen synthesis and breakdown.***
indicates activation *indicates inhibition* *indicates the effect of kinases* *indicates the effect of phosphatases*

a high affinity for UDPG and glucose-6-phosphate. High concentrations of ATP (and possibly other nucleotides) inhibit the activities of both the *a* and *b* forms of the synthetase by competing for the UDPG. This inhibition can be reversed by glucose-6-phosphate and magnesium ions (Gold, 1970).

Regulation of Enzymes involved in Glycogen Synthesis and Breakdown

The glycogen stored in the liver is used as a reserve of glucose for the blood while muscle glycogen is a glycolytic fuel for the supply of ATP in anaerobic conditions. It is not surprising, therefore, to find the metabolism of liver glycogen tightly regulated by the level of glycemia and by several hormones according to the general demand while that of muscle glycogen is much more under the control of local factors. While the active form of glycogen synthetase is the dephosphorylated form, that of glycogen phosphorylase is the phosphorylated form. Most of the factors which influence the activation of glycogen synthetase also have a profound effect on the inactivation of glycogen phosphorylase. Although the mechanism of regulation by metabolites and hormones may be independent of one another, a balance is always maintained.

Effect of Glucose and Glycogen

The concentration of glucose in the blood has been shown to control both the degradation and synthesis of glycogen in the liver. The increase in the rate of glycogen synthesis due to glucose is not the result of an increase in the concentration of intermediary metabolites in the metabolic pathway leading to glycogen synthesis. On the contrary, there is a decrease in the concentration of UDPG and glucose-6-phosphate. The effect is due to a conversion of the synthetase into its active form (De Wulf & Hers 1967).

When a glucose load is given intravenously, the rate of glycogen synthesis starts to increase only after a short latency, indicating that the effect of glucose on the activity of the synthetase is indirect. As this effect has been obtained in animals made diabetic by the administration of anti-insulin serum, it seems not to be mediated by insulin (Hers et al. 1970). The specific stimulation of liver glycogen phosphorylase phosphatase by glucose *in vitro* has been observed by Stalmans et al. (1970). This observation could very well explain the *in vivo* lowering of liver glycogen phosphorylase activity in animals treated with glucose (De Wulf & Hers 1968).

Phosphorylase *a* has also been shown to be a powerful inhibitor of glycogen synthetase phosphatase (Stalmans et al. 1971). Phosphorylase *b* is much less inhibitory. The activation *in vivo* of glycogen synthetase by glucose is therefore explained by the increased activity of phosphorylase phosphatase. Once phosphorylase is inactivated, the synthetase phosphatase now released from inhibition can activate glycogen synthetase.

Hyperglycemia can, therefore, bring about re-

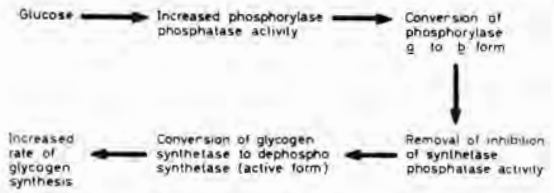

Fig 3 Effect of glucose on Glycogen synthesis

duced glycogenolysis and increased glycogensis — an effect not mediated by insulin.

The effect of glycogen concentration on the rate of glycogen synthesis has not been well investigated. Larner (1967) has explained the inverse relationship between glycogen content in the muscle and the percentage of synthetase in the active form on the basis of inhibition of synthetase phosphatase by glycogen. This could again be an indirect effect exerted through the action of glycogen on phosphorylase phosphatase which has been shown to be inhibited by glycogen *in vitro* (De Wulf et al. 1970).

The Effect of Hormones

The role played by hormones in the regulation of glycogen metabolism is also interesting. Insulin, when given together with glucose, has been shown to cause glycogen deposition in the liver. This effect has been attributed to an activation of glycogen synthetase (Bishop & Larner 1967). Insulin alone, however, does not induce an activation of glycogen synthetase but it has been shown that the synthesis of the inducible enzyme glucokinase is dependent on insulin (Salas et al. 1963). Is it also likely that insulin may activate the phosphodiesterase and thereby regulate the amount of cyclic 3,5-AMP present within the cells? A reduction in the amount of cyclic AMP would cause both an increase in the level of glycogen synthetase *a* and a decrease in that of phosphorylase *a*, resulting in increased glycogen production. At the present time however, it is not known for certain how insulin might influence any of the regulatory mechanisms.

The action of the hormones, which are antagonistic to insulin, namely adrenaline and glucogon, has been well studied. These hormones are known to affect glycogen metabolism by changes in tissue concentration of cyclic 3,5-AMP. The levels of active phosphorylase and of active synthetase are controlled by the opposing effects of the kinases and phosphatases. In muscle, and probably in liver, the same protein kinase acts as a phosphorylase kinase-kinase and as a synthetase kinase.

This protein kinase is cyclic AMP dependent and its action inhibits glycogen synthesis and promotes glycogenolysis. This would lead to hyperglycamia which, in turn, would start a series of changes via phosphorylase phosphatase, phosphorylase *a* and synthetase phosphatase to bring about increased glycogen synthesis and lowered glycogenolysis. The actual amounts of active phosphorylase or active synthetase in a tissue is the result of a balance between phosphorylation and dephosphorylation. 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. In the latter case, the effect is produced by increased *de novo* synthesis of the phosphorylase phosphatase enzyme.

The action of the glycogen metabolism regulators on the kinases and phosphatases in maintaining the regulation are strikingly similar. It should be noted that the phosphorylase kinase is an allosteric protein and the mechanism of the regulation could be due to the allosteric properties of the enzyme (Madsen, 1964). Black and Wang (1968) have described a mechanism whereby the activation of the phosphorylase *b* is due to a two-stage allosteric transition of the enzyme protein, involving the affinity of the enzyme to its substrate and its catalytic efficiency.

Other Enzymes, Hormones and Tissue Differences involved in the Regulation of Glycogen Metabolism

Other enzymes also take part in the regulation of glycogen metabolism. The relatively irreversible action of phosphofructokinase in converting fructose-6-phosphate to fructose 1,6-diphosphate has long been recognised as a regulatory mechanism. This enzyme is strongly inhibited allosterically by high concentrations of ATP, thereby preventing further production of this nucleotide by glycogen breakdown.

Hormones, such as ACTH, the adrenocortico steroids and thyroid hormone, all of which promote glycogen breakdown, may affect glycogen metabolism by either destroying insulin or by producing different end organ responses. Hormonal effects also vary from one tissue to another. Glucagon, a well-known example, has an effect on liver phosphorylase but has little or no effect on muscle phosphorylase.

Finally, it should be realised that the pattern of glycogen metabolism may itself vary in the different tissues. Aduoury (1969) has shown that in fasting animals, there was an increase in cardiac muscle glycogen while the glycogen content in skeletal muscle remained constant. In skeletal mus-

cle, there was a decrease in phosphorylase activity on fasting while the glycogen synthetase and other regulators were unaffected. The only change in cardiac muscle at fasting was an increase in the D-form of glycogen synthetase.

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