The Usefulness of 'Futile Cycles'

H. G. HERS

Laboratoire de Chimie Physiologique, Université Catholique de Louvain, and
International Institute of Cellular and Molecular Pathology, UCL 7539,
avenue Hippocrate 75, B-1200 Brussels, Belgium

General considerations

A 'futile cycle' is a cyclic transformation of which the net balance is the hydrolysis of
ATP to ADP and P_i. Many biochemists react almost emotionally against such a deliberate
waste of ATP. There is, however, more and more evidence that several so-called futile
cycles are actually operating in the animal body; it is therefore expected that they play
some useful role.

Some of these cycles provide the cell with a valuable regulatory mechanism. This is
particularly apparent when recycling occurs at the level of a metabolic crossroad, such as
glucose 6-phosphate or phosphoenolpyruvate, where it participates in the control of the
metabolic flow. The advantage of recycling must also be considered at the level of the
entire organism. The animal body is indeed a complex organization in which the most
sophisticated organ is the brain. Yet the brain has a specific requirement for glucose, and
one major role of the liver is to prevent hypoglycaemia from occurring. The expense of a
few ATP molecules appears a trivial consideration when compared with the vital
importance of this function. It is indeed remarkable that the Cori cycle is a futile cycle
and that gluconeogenesis operates even in the fed animal, although under this condition
glucose is abundant. This indicates that the body does not take any risk of being de-
prived of glucose and this seems particularly wise in organisms that have a body
temperature of 37°C and that need continuous formation of heat to maintain this body
temperature.

I will briefly discuss below the main futile cycles that operate in carbohydrate metabo-
lism in the liver and discuss their general organization and their possible usefulness to
the liver cell.

Glycogen synthesis and breakdown

As reviewed elsewhere (Hers, 1976), two mechanisms prevent any important synthesis
of glycogen to occur in the liver when glycogen degradation is in operation: (1) the same
cyclic AMP-dependent protein kinase inactivates glycogen synthetase and causes the
activation of glycogen phosphorylase; (2) phosphorylase a is a potent inhibitor of
synthetase phosphatase, the enzyme that activates glycogen synthetase and whose
activity initiates glycogen synthesis. However, the system is somewhat disorganized in
the fasting animal, in that glycogen synthetase is partially activated even in the presence
of a large amount of phosphorylase a. It seems therefore that in the fasting animal some
recycling between glucose 1-phosphate and glycogen is in operation. The probable
advantage of this recycling is to protect the glycogen molecule against a complete
degradation by phosphorylase and to avoid the disappearance of the primer, which will
be necessary for glycogen synthesis when glucose is available again.

Glucose/glucose 6-phosphate recycling

The demonstration that, when isolated hepatocytes are incubated with [U-14C,
2-3H]glucose, the yield of 3H2O is two to three times that of 14C utilization (Clark et al.,
1973), is the best proof that glucokinase and glucose 6-phosphatase operate simulta-
naneously. It has been estimated that about 1 μmol of substrate is recycled/min per g
of liver in normal conditions (Hue & Hers, 1974a).

The significance of the glucose/glucose 6-phosphate recycling was first discussed by
Cahill et al. (1959), who pointed out that 'two separate enzymatic reactions controlling
a given step, but in opposing directions, can change not only the rate but also the direc-
tion of net flow as well as the final ratio of the two substrates when a steady state is achieved. When one (or both) of these enzymes is under hormonal control, that pathway of metabolism loses its autonomy and is subjected to distant control. Therefore, this comment is still valid, it must be pointed out that no rapid hormonal control of glucokinase or glucose 6-phosphatase has ever been described and that the rate of each of these reactions is determined only by the availability of substrate.

An attempt to explain how the cycle operates was made by Newsholme & Gevers (1967) and, in greater detail, by Newsholme & Start (1973); these authors correctly pointed out that a small change in glucose concentration, from 4 mM to 6 mM, is enough to produce a complete change in the direction of glucose metabolism in the liver. They assumed that only the activity of glucokinase is affected by the change in glucose concentration and that the activity of glucose 6-phosphatase and the concentration of its substrate remain fairly constant. By contrast, it has been pointed out by Hue & Hers (1974) that the concentration of glucose 6-phosphate in the liver and the rate of its hydrolysis vary to a large extent. These variations are related to glycogen metabolism and to gluconeogenesis, and they play a major role in the control of glucose uptake and output by the liver. Indeed, glycogen synthesis is controlled by a pull mechanism in which the activation (namely by glucose) of the last enzyme of the pathway, glycogen synthetase, causes an important decrease in the concentration of intermediary metabolites, UDP-glucose and glucose 6-phosphate (see Hers, 1976). Fig. 1 illustrates that because of these changes in glucose 6-phosphate concentration, the stimulation of glucose uptake by an elevation of the concentration of glucose is much larger than the stimulation of glucokinase. By contrast, glycogenolysis is controlled by a push mechanism in which the activation of glycogen phosphorylase is responsible for an increase in the concentration of glucose 6-phosphate. A similar situation exists in the case of gluconeogenesis (see below).

It therefore appears that the major advantage of the glucose/glucose 6-phosphate recycling is to allow large changes in glucose uptake or output controlled by the concentration of substrates only. This allows an automatic adaptation of the flux of glucose not only to the rate of glycogen synthesis and breakdown but also to the rate of glycolysis, gluconeogenesis and pentose phosphate pathway. The alternate mechanism would be an on/off control of the activity of glucokinase and glucose 6-phosphatase, which would necessarily be extremely complex because it ought to be adapted to several important metabolic pathways.

Fructose 6-phosphate/fructose diphosphate recycling

There is still great doubt concerning the importance of this recycling in the normal liver. As discussed by Dr. Hue in this colloquium (Hue, 1976), the evidence based on the conversion of [5-3H]glucose into H₂O is subjected to criticism. On the other hand, the fact that [1-14C]glucose (Cook & Lorber, 1952; Hers, 1955) and [1-14C]galactose (Topper & Stetten, 1951) are converted into [1-14C]glycogen in the liver with little contamination on other carbons is an argument against any important recycling between fructose 6-phosphate and fructose diphosphate.

Pyruvate/phosphoenolpyruvate recycling and the control of gluconeogenesis

This cycle includes three reactions catalysed by pyruvate carboxylase, phosphoenolpyruvate carboxykinase, and pyruvate kinase. One ATP molecule is consumed in each of the two first reactions and one ATP molecule is formed in the last step. The first indication that this recycling occurs in normal liver was given by Friedmann et al. (1971) who showed that [2-14C]pyruvate is progressively converted into [2,3-14C]-pyruvate.

Control of this cycle could occur at each of the three steps. Indeed, pyruvate carboxylase, which is an intramitochondrial enzyme, could be regulated by the concentration of acetyl-CoA (Utter & Scrutton, 1969) and also possibly by other effectors; phosphoenolpyruvate carboxykinase is an adaptive enzyme whose concentration in the liver is
Fig. 1. Influence of the concentration of glucose and of glucose 6-phosphate on the net flux of glucose to glucose 6-phosphate

The upper curve represents the activity of glucokinase, assuming a $V_{\text{max}}$ of 2.5 $\mu$mol/min per g and a $K_{m}$ of 10 mM. The other curves have been obtained by subtracting the activity of glucose 6-phosphatase, for which a $V_{\text{max}}$ of 10 $\mu$mol/min per g and a $K_{m}$ of 2 mM have been adopted. At 0.2 mM-glucose 6-phosphate, considered as physiological, the rate of hydrolysis is close to 0.9 $\mu$mol/min per g. It appears that at 5.5 mM-glucose (100 mg/100 ml), although glucose is phosphorylated at a rate of 0.88 $\mu$mol/min per g (point a), there is a slight output of glucose (point c). At the same concentration, glucose uptake occurs if glucose 6-phosphate concentration is lowered (point c), as results for instance from the activation of glycogen synthase by glucocorticoids. If glucose concentration is raised to 11 mM without changing glucose 6-phosphate concentration (point f), there is an uptake of 0.41 $\mu$mol/min per g, whereas, if glucose 6-phosphate concentration is simultaneously raised to 0.3 mM, the uptake remains close to zero (point g). Similar conditions have been observed in the first few minutes after a glucose load, before activation of glycogen synthetase had occurred. Later on, glucose 6-phosphate concentration is lowered by about 50%; the glucose uptake is now 0.86 $\mu$mol/min per g (point d), reaching then 66% of the rate of glucose phosphorylation by glucokinase (point b). The dotted arrows show the change of glucose flux due to a decrease in glucose 6-phosphate concentration with (point d) and without (point c) increase in glucose concentration. (From Hue & Hers, 1974b)

increased after administration of glucocorticoids, fasting or induction of diabetes, and decreased after surrenalectomy (Shrago et al., 1963). Concentration of pyruvate kinase can also vary to a great extent according to dietary conditions, being increased when the animals are fed a high carbohydrate diet and decreased on fasting (Krebs & Eggleston, 1965). A rapid regulation of the latter enzyme occurs through phosphorylation and dephosphorylation by cyclic AMP protein kinase, as has been demonstrated in a reconstructed system (Ljungström et al., 1974; Titanji et al., 1976) and also in isolated rat hepatocytes (Feliu et al., 1976). In the latter preparation the stimulation of gluconeogenesis by glucagon is inversely correlated to the inactivation of pyruvate kinase, and the phosphorylation of this enzyme is at present the only known molecular mechanism by which glucagon stimulates gluconeogenesis.
The rate of gluconeogenesis in the liver is greatly influenced by the concentration of lactate and pyruvate (Exton & Park, 1967) and its control may be considered as a push mechanism. Accordingly, the control of gluconeogenesis by the activity of pyruvate kinase is necessarily also a push mechanism in which the effect of pyruvate kinase activity is mediated by a change in the concentration of phosphoenolpyruvate in the cell, and this concentration is indeed known to be increased by glucagon and by cyclic AMP (Exton & Park, 1969).

When gluconeogenesis predominates over glycolysis, the concentration of phosphoenolpyruvate is the result of a balance between an input via phosphoenolpyruvate carboxykinase and an output via enolase and pyruvate kinase. Because the latter enzyme displays a sigmoidal saturation curve for its substrate, a control mechanism based on its inactivation is expected to be less efficient at low concentrations of phosphoenolpyruvate. Such a low concentration could be found when the activity of pyruvate kinase has been increased by a high carbohydrate diet or when the activity of phosphoenolpyruvate carboxykinase is low, as after surrenalectomy. These kinetics prevent a complete disappearance of phosphoenolpyruvate and explain why gluconeogenesis is never completely cancelled, even when glucose is abundant. It could also explain why, as reported by Exton et al. (1970), surrenalectomized animals are poorly reactive to the stimulatory action of glucagon.

The advantage of the pyruvate-phosphoenolpyruvate cycle seems again to be an economy of regulatory mechanisms at an important crossroad of metabolic pathways. Indeed, phosphoenolpyruvate needs to be converted into pyruvate when glycolysis is intense, as for instance after a load of fructose or under anaerobic conditions. In other circumstances, gluconeogenesis predominates and needs itself to be regulated; one advantage of having this regulation at the level of pyruvate kinase is to have only one regulatory mechanism common to glycolysis and gluconeogenesis and not to need a complex co-ordination between several points of control.

Topper, Y. J. & Stetten, D., Jr. (1951) J. Biol. Chem. 193, 149–155