Difference in glucose sensitivity of liver glycolysis and glycogen synthesis

Relationship between lactate production and fructose 2,6-bisphosphate concentration

Louis HUE, Francisco SOBRINO* and Lisardo BOSCA

Hormone and Metabolic Research Unit, University of Louvain and International Institute of Cellular and Molecular Pathology, UCL 7529, Avenue Hippocrate 75, B-1200 Brussels, Belgium

(Received 20 June 1984/Accepted 30 August 1984)

1. Incubation of isolated rat hepatocytes from fasted rats with 0–6 mM-glucose caused an increase in [fructose 2,6-bisphosphate] (0.2 to about 5 nmol/g) without net lactate production. A release of $^3\text{H}_2\text{O}$ from $[3\text{-}^3\text{H}]\text{glucose}$ was, however, detectable, indicating that phosphofructokinase was active and that cycling occurred between fructose 6-phosphate and fructose 1,6-bisphosphate. A relationship between [fructose 2,6-bisphosphate] and lactate production was observed when hepatocytes were incubated with $[\text{glucose}] > 6 \text{ mM}$. Incubation with glucose caused a dose-dependent increase in [hexose 6-phosphates]. 2. The maximal capacity of liver cytosolic proteins to bind fructose 2,6-bisphosphate was 15 nmol/g, with affinity constants of 5×10^6 and $0.5 \times 10^6 \text{ M}^{-1}$. One can calculate that, at $5 \mu\text{M}$, more than 90% of fructose 2,6-bisphosphate is bound to cytosolic proteins. 3. In livers of non-anaesthetized fasted mice, the activation of glycogen synthase was more sensitive to glucose injection than was the increase in [fructose 2,6-bisphosphate], whereas the opposite situation was observed in livers of fed mice. Glucose injection caused no change in the activity of liver phosphofructokinase-2 and decreased the [hexose 6-phosphates] in livers of fed mice.

In liver, glucose is known to stimulate both glycolysis and glycogen synthesis. The stimulation of glycogen synthesis results from an activation of glycogen synthase preceded by an inactivation of glycogen phosphorylase (for reviews, see Stalmans, 1976; Hers, 1976). The effect of glucose to stimulate liver glycolysis is best explained by fructose 2,6-bisphosphate, the most sensitive stimulator of phosphofructokinase. The stimulation of glycolysis by glucose in hepatocytes results from an amplification mechanism exerted by a glucose-induced rise in [fructose 6-phosphate] (Hue *et al.*, 1981), which is the substrate of phosphofructokinase-2, and an inhibitor of fructose 2,6-bisphosphatase (Hers & Van Schaftingen, 1982).

However, in the livers of conscious animals, [glucose 6-phosphate] is known to decrease after a glucose load (De Wulf & Hers, 1967; Hue & Hers, 1974), and one may wonder whether this decrease

in hexose 6-phosphates would be reflected in [fructose 2,6-bisphosphate] *in vivo*. The glucose effect *in vivo* would then be different from that in hepatocytes. In addition, since glucose is known to influence both glycogen synthesis and glycolysis, it would be of interest to know whether there is a difference in sensitivity for glucose between glycolysis and glycogen synthesis. Experiments were carried out to study these problems and are reported here. The activity of glycogen synthase *a* was chosen as the parameter for glycogen synthesis, and [fructose 2,6-bisphosphate] and lactate production were chosen for glycolysis. The relationship between the rate of liver glycogen synthesis *in vivo* and the activity of glycogen synthase *a* is well established (De Wulf & Hers, 1968b). Such a relationship between [fructose 2,6-bisphosphate] and lactate production in hepatocytes is less clear. Previous results have indicated (Hue, 1982a) that the relation is valid in hepatocytes from fed rats; however, in hepatocytes from fasted rats incubated at [glucose] less than 10 mM, there was little, if any, net lactate production despite the presence of fructose 2,6-bisphosphate. These conditions have

* Permanent address: Catedra de Bioquímica, Facultad de Medicina, Universidad Sevilla, Avda Sanchez Pijuan 4, Sevilla 9, Spain.

been further studied and the results are presented below.

Materials and methods

Incubation of hepatocytes and experiments with mice

Hepatocytes from overnight-fasted rats were prepared as described previously (Hue *et al.*, 1978). After 20 min of incubation at 37°C in the presence of O₂/CO₂ (19:1), glucose was added to the cell suspension (2 ml, containing about 100 mg of cells). The incubation was continued for 30 min and the reaction was stopped by the addition of 0.5 ml of ice-cold 2M-HClO₄ to the cell suspension. For the measurement of fructose 2,6-bisphosphate, samples (0.1 ml) were taken after 15 min of incubation, frozen in acetone/solid CO₂ and further processed as described below. For the measurement of ³H₂O release from [3-³H]glucose, the radioactive glucose (Amersham International, Amersham, Bucks., U.K.) was added to the cell suspension (0.5 µCi/ml) and ³H₂O was separated from the radioactive glucose in neutralized HClO₄ extracts, as described previously (Bontemps *et al.*, 1978).

Non-anaesthetized mice (Naval Medical Research Institute; 25–30 g body wt.) were injected intravenously (1 ml/100 g body wt.) with different doses of glucose or with 0.15M-NaCl (control mice). The mice were killed by decapitation 15 min after the injection and their livers were quick-frozen (Wollenberger *et al.*, 1960) within 5 s.

Measurement of metabolites and fructose 2,6-bisphosphate

Glucose 6-phosphate (Hohorst, 1963a) and lactate (Hohorst, 1963b) were measured enzymically in neutralized HClO₄ extracts as indicated. For the measurement of fructose 2,6-bisphosphate, 0.1 ml samples of frozen cell suspension or 100 mg samples of frozen liver were homogenized in 50 mM-NaOH and kept at 80°C for 10 min. The extracts were cooled and neutralized by the addition of 25 mM-acetic acid and 0.1 M-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid], pH 8, and centrifuged for 5 min in an Eppendorf Microfuge. Fructose 2,6-bisphosphate was measured in the supernatant as described by Van Schaftingen *et al.* (1982b).

Measurement of the activity of phosphofructokinase-2 and glycogen synthase a

Cyclic-AMP-dependent protein kinase phosphorylates and inactivates phosphofructokinase-2 and activates fructose 2,6-bisphosphatase (Hers & Van Schaftingen, 1982). In control rat liver, the 'active' form of phosphofructokinase-2 (measured at pH 6.6 in the presence of 1 mM-fructose 6-

phosphate) is about equal to the total activity (measured at pH 8.5 with 5 mM-fructose 6-phosphate), whereas in glucagon-treated livers, the 'active' form represents less than 10% of the 'total' form (Bartrons *et al.*, 1983). In mouse liver, we observed that the total activity was about half of that in rat liver, and the 'active' form remaining after glucagon treatment represented 40–50% of the 'total' form. Frozen liver samples were homogenized in 10 vol. of a buffer containing 0.1 M-KCl/20 mM-potassium phosphate/10 mM-EDTA, pH 7. Phosphofructokinase-2 was measured at pH 6.6 and 8.5 as described previously (Bartrons *et al.*, 1983). For the measurement of glycogen synthase a, frozen liver samples were homogenized and the activity was measured at 25°C as described previously (Hue *et al.*, 1975).

Binding measurements

Rat liver homogenates were prepared in 100 mM-KCl/5 mM-MgCl₂/1 mM-EDTA/1 mM-dithioerythritol/5 mM-potassium phosphate/50 mM-Hepes, pH 7.2, and centrifuged at 30000g for 30 min. A high-speed supernatant (100000g for 1 h) was filtered through 20 vol. of Sephadex G-25 equilibrated with the homogenization medium. Binding was measured in 1 ml of the same medium containing appropriate [fructose 2,6-bisphosphate], and 0.1 mM-fructose 6-phosphate and 0.3 mM-glucose 6-phosphate to prevent unspecific binding and to inhibit fructose 2,6-bisphosphatase activity (Van Schaftingen *et al.*, 1982b). After 5 min of incubation at 23°C, 0.3 ml of the mixture was filtered through a UH 100/10 dialysis bag fitted on a vacuum line (Schleicher and Schüll, Dassel, Germany). After 3–4 min, samples were taken to measure [fructose 2,6-bisphosphate] in the ultrafiltrate, whereas the total [fructose 2,6-bisphosphate] was measured in samples taken from the dialysis bag. The binding of fructose 2,6-bisphosphate to the cytosolic proteins was measured at equilibrium because, after 5 min of incubation, free [fructose 2,6-bisphosphate] was stable and [hexose 6-phosphates] were the same in both compartments.

Results

Relationship between lactate production and [fructose 2,6-bisphosphate]

Hepatocytes from fasted rats were incubated with various [glucose] including concentrations less than 10 mM. Increases in lactate production, [fructose 2,6-bisphosphate] and the production of ³H₂O from [3-³H]glucose were measured; the results are shown in Fig. 1. The deitritiation of [3-³H]glucose occurs at the level of triose phosphates, and in the pentose phosphate cycle (Katz &

Fig. 1. Effect of glucose on lactate production (Lactate), $^3\text{H}_2\text{O}$ production from $[3\text{-}^3\text{H}]\text{glucose}$ (Glucose) and [fructose 2,6-bisphosphate] (Fru-2,6- P_2) in hepatocytes from fasted rats

Values shown are means for four to six different cell preparations. Bars represent S.E.M. The production of $^3\text{H}_2\text{O}$ was measured in one cell preparation.

Fig. 2. Binding of fructose 2,6-bisphosphate to liver cytosolic proteins

The binding of fructose 2,6-bisphosphate to 19 mg of cytosolic proteins from liver of fed rats was measured in the presence of 5 mM- MgCl_2 and at different concentrations of total fructose 2,6-bisphosphate. Values shown are means for three separate experiments; bars represent S.E.M. Insert shows Scatchard plots of the same data: r represents pmol of fructose 2,6-bisphosphate bound/mg of cytosolic protein (1 g of liver contains 100 mg of cytosolic protein).

Rognstad, 1976; Hue, 1981). Since the activity of the pentose phosphate cycle and lipogenesis are negligible at low [glucose] and in livers of fasted rats (Clark *et al.*, 1974), the rate of deamination of $[3\text{-}^3\text{H}]\text{glucose}$ gives a reasonable estimation of the flux through phosphofructokinase-1.

Incubations of hepatocytes with glucose concentrations above 6 mM resulted in a parallel increase in the three parameters measured. The rate of glucose deamination was always larger than the rate of lactate production expressed as glucose units consumed. At [glucose] below or equal to 6 mM, no increase in lactate production could be detected, despite the presence of up to 5 nmol of fructose 2,6-bisphosphate/g and, at 10 mM-glucose, less than 0.1 μmol of glucose was converted into CO_2 /30 min per g of cells (results not shown). This could result either from extensive recycling between glucose and triose phosphates or from the binding of fructose 2,6-bisphosphate to cytosolic protein, or from both. Fig. 1 shows that at low [glucose], when no lactate was produced, glucose was deaminated to some extent, indicating that phosphofructokinase-1 was active and that cycling occurred under these conditions.

Binding of fructose 2,6-bisphosphate to cytosolic proteins

Since [fructose 2,6-bisphosphate] is about μM , i.e. in the same range as some [proteins], its 'free' concentration could depend on its binding to proteins such as fructose 1,6-bisphosphatase, phosphofructokinase-1 and -2 and perhaps others. The binding of fructose 2,6-bisphosphate to cytosolic proteins was therefore measured. The non-linear Scatchard plot obtained (Fig. 2) suggests 'negative co-operativity' (less affinity as occupancy increases), or the presence of two or more classes of binding sites. The latter is probably true, since fructose 2,6-bisphosphate is known to bind to more than one protein in the cytosol. From the data of Fig. 2, one can estimate that the maximal binding capacity of the liver for fructose 2,6-bisphosphate is 13–15 nmol/g of liver and that the affinity constants for the highest- and the lowest-affinity sites are about 5×10^6 and $0.5 \times 10^6 \text{ M}^{-1}$ respectively. The binding of fructose 2,6-bisphosphate to cytosolic proteins was not detectable in the absence of Mg^{2+} and in the presence of 5 mM-EDTA.

Effect of glucose injection on [fructose 2,6-bisphosphate] and glycogen synthase α activity in mouse liver

These experiments were carried out with conscious mice rather than with non-aesthetized rats, since mice are easier to handle in large numbers. The time course of the changes in liver fructose 2,6-bisphosphate and synthase α was first

studied in mice given 1 mg of glucose/g body wt. The activation of synthase and the rise in fructose 2,6-bisphosphate, when observed, were complete within 15 min after the injection (results not shown).

The glucose sensitivity was further analysed, and results are shown in Figs. 3(a) and 3(b). A significant rise in [fructose 2,6-bisphosphate] was observed in livers of fed mice given 0.5 mg of glucose/g, whereas at least 1 mg/g was required to induce a detectable change in [fructose 2,6-bisphosphate] in the livers of fasted mice. The maximal values obtained in fasted mice injected with 5 mg of glucose/g were different from the values in fed mice; however, when larger doses of glucose were given (7.5 mg/g) the values were not significantly different, and were about 20 nmol of fructose 2,6-bisphosphate/g of liver (results not shown). In contrast, the activation of glycogen synthase in fasted mice required less glucose than in fed mice. The maximal activation obtained was not significantly different. These results suggest that the sensitivity of livers to glucose depends on the nutritional state of the animal, and is not the same for the activation of glycogen synthase and for the increase in [fructose 2,6-bisphosphate].

Activity of phosphofructokinase-2 and concentration of hexose 6-phosphates

The 'active' form of phosphofructokinase-2 was measured in liver homogenates from fed and fasted mice which were given different doses of glucose. The activity at pH 6.6 was 1.72 ± 0.23 ($n = 8$) and

0.76 ± 0.07 ($n = 4$) nmol/min per g in the livers of fed and fasted mice respectively, and it was not modified after the injection of glucose.

The concentration of hexose 6-phosphate(s) was also measured in the livers of mice injected with glucose. Table 1 shows that glucose induced a decrease in [hexose 6-phosphates] in the livers of conscious fed mice, whereas no difference was observed in livers of fasted mice, or when the mice were anaesthetized (results not shown). In non-anaesthetized fed mice, 15 min after the injection, the glycaemia returned to control basal values when the doses injected were > 1 mg/ml, whereas, with higher doses, the glycaemia was increased. In any case, no correlation between [glucose] and [hexose 6-phosphates] was obtained (results not shown). In contrast, a glucose-dependent increase in [hexose 6-phosphates] was observed in isolated hepatocytes incubated with glucose (Table 1).

Discussion

Metabolic role of fructose 2,6-bisphosphate

Our results clearly demonstrate that the relationship between [fructose 2,6-bisphosphate] and the production of lactate holds only at high [fructose 2,6-bisphosphate], above 5 nmol/g. Below this threshold the production of lactate and CO_2 is negligible. It is indeed well known that glucose utilization and net glycolysis are not detectable in livers from starved rats supplied with low [glucose] (Woods & Krebs, 1971; Brunengraber *et al.*, 1973; Seglen, 1974; Katz *et al.*, 1975). However, phos-

Fig. 3. Effect of intravenous glucose load on the concentration of fructose 2,6-bisphosphate (a) and on the activity of glycogen synthase a (b) in livers of fasted and fed mice

Non-anaesthetized mice were injected intravenously with different doses of glucose and were killed 15 min after the injection. Values shown are means and bars represent S.E.M. The numbers of mice are 8–16 for [glucose] ≤ 1 mg/g and 4–8 for [glucose] < 1 mg/g. *Denotes values that are statistically different from the values of untreated controls ($P < 0.05$).

Table 1. *Effect of glucose on the concentration of hexose 6-phosphates in livers of fasted and fed mice, and in isolated hepatocytes from fasted rats*

Experimental procedure was as in Fig. 1 for hepatocytes, and as in Fig. 3 for mice. Values shown are means $\pm$ S.E.M. for the numbers of animals or experiments shown in parentheses. Hexose 6-phosphates are the sum of glucose 6-phosphate and fructose 6-phosphate.

Glucose injected into mice (mg/g body wt.)	Concn. of hexose 6-phosphates (nmol/g of liver)	
	Fed mice	Fasted mice
0	201 $\pm$ 22 (8)	104 $\pm$ 17 (8)
0.25	95 $\pm$ 13 (4)	
0.50	92 $\pm$ 44 (6)	
0.75	97 $\pm$ 27 (4)	
1	97 $\pm$ 17 (8)	104 $\pm$ 20 (10)
2	85 $\pm$ 26 (5)	
5	54 $\pm$ 12 (4)	
Glucose in the incubation medium (mM)		
	Fasted rats	
0	55 $\pm$ 3 (4)	
5	72 (2)	
10	106 $\pm$ 6 (4)	
20	134 $\pm$ 14 (4)	
40	228 $\pm$ 30 (4)	

Table 2. *Fructose 2,6-bisphosphate-binding sites in rat liver cytosol*

References: a, Riou *et al.* (1977); b, McGrane *et al.* (1983); c, Pilkis *et al.* (1981); d, Kitajima & Uyeda (1983); e, Reinhart & Lardy (1980); f, Van Schaftingen *et al.* (1981); g, Sakakibara *et al.* (1984); h, Van Schaftingen *et al.* (1982a).

Enzyme	Specific activity (units/mg of protein)	Liver content (units/g)	Subunit		K_m , K_a or K_i (μ M)	References
			No. of subunits; M_r of subunit	concn. (μ M)		
Fructose 1,6-bisphosphatase	30	15	4; 41000	12	0.5–5	a,b,c,d
Phosphofructokinase-1	85	2	4; 80000	0.3	0.1	e,f
Phosphofructokinase-2/ fructose 2,6-bisphosphatase	0.057	0.0025	2; 55000	0.8	0.2	g,h

Σ binding sites = approx. 13 μ M.
Cytosolic maximal binding capacity = 13–15 nmol/g.

phofructokinase is active, as evidenced by the production of $^3\text{H}_2\text{O}$ from $[3\text{-}^3\text{H}]\text{glucose}$, and hence cycling should occur between fructose 6-phosphate and fructose 1,6-bisphosphate. A similar situation is observed when hepatocytes are incubated with low [dihydroxyacetone] or [fructose] (Hue & Bartrons, 1984). Therefore, at low [fructose 2,6-bisphosphate], both phosphofructokinase-1 and fructose 1,6-bisphosphatase are active. When [fructose 2,6-bisphosphate] is further increased, fructose 1,6-bisphosphatase becomes progressively inhibited and lactate is then produced. However, the inhibition is not complete, and cycling persists (Van Schaftingen *et al.*, 1980a,b). Consequently, the physiological significance of fructose 2,6-

bisphosphate is best expressed at relatively high concentrations, when it allows the pathway to switch from gluconeogenesis to glycolysis, and vice versa.

Binding of fructose 2,6-bisphosphate

Table 2 summarizes our results together with published data on the concentration and the kinetic constants for fructose 2,6-bisphosphate of phosphofructokinase-1 and -2, fructose 2,6-bisphosphatase and fructose 1,6-bisphosphatase in rat liver. It also appears that the sum of the binding capacities of these enzymes accounts for more than 90% of the maximal binding capacity of the cytosol. It seems very unlikely that many other

proteins present in large quantity would contribute to the binding of fructose 2,6-bisphosphate.

From the data given in Table 2, the proportion of fructose 2,6-bisphosphate that is free or bound to each enzyme at different total [fructose 2,6-bisphosphate] can be calculated and is shown in Fig. 4. Fructose 2,6-bisphosphate binds first to phosphofructokinase-1 and -2, and more than 90% of fructose 2,6-bisphosphate is bound when its total concentration is $5 \mu\text{M}$. Comparison of Fig. 1 with Fig. 4 also shows that there is a remarkable correlation between the calculated free [fructose 2,6-bisphosphate] and the production of lactate measured in hepatocytes. It is noteworthy that the apparent K_D values of phosphofructokinase-1 and -2 for fructose 2,6-bisphosphate (Fig. 4) are at least one order of magnitude greater than the known kinetic constants (Table 2). This shift in apparent affinity is mainly due to the high molarity of fructose 1,6-bisphosphatase.

Difference in glucose sensitivity of liver glycolysis and glycogen synthesis

Our results indicate that there is a difference in

Fig. 4. Calculated binding of fructose 2,6-bisphosphate to phosphofructokinase-1 (PFK-1) and -2 (PFK-2) and to fructose 1,6-bisphosphatase (FBPase 1), and concentration of free fructose 2,6-bisphosphate

The saturation of phosphofructokinase-1 and -2 and of fructose 1,6-bisphosphatase by fructose 2,6-bisphosphate, and the concentration of free fructose 2,6-bisphosphate, were calculated from enzyme molarity and K_D values which were assumed to be close to kinetic constants given in Table 2. K_A values of 1×10^6 , 10×10^6 and $5 \times 10^6 \text{ M}^{-1}$ were chosen for fructose 1,6-bisphosphatase, phosphofructokinase-1 and phosphofructokinase-2/fructose 2,6-bisphosphatase, respectively.

the effect of glucose on the activation of glycogen synthase and on the increase in [fructose 2,6-bisphosphate] in mouse liver *in vivo*. Experiments with hepatocytes from fasted rats have also shown that synthase activation starts indeed at lower [glucose] than in hepatocytes from fed rats (Hue *et al.*, 1975). Since, within certain limits, synthase activity and [fructose 2,6-bisphosphate] give a reasonable estimation of the rate of glycogen synthesis and lactate release, it follows that the sensitivity of the two pathways for glucose is different *in vivo* and that this depends on the nutritional state of the animal. This interpretation is valid only as far as the conclusions drawn from experiments on rat hepatocytes also apply to mouse liver.

The physiological meaning of this observation is the following. In the fasted state, the glycogen stores are first replenished after glucose administration, and it is only when an excess of glucose is given that it is glycolysed and possibly transformed into fatty acids. When the liver capacity to synthesize glycogen is saturated or when the glycogen stores are replenished, a glucose load can only be glycolysed and/or recycled into glucose. Both processes actually occur in the fed state (Van Schaftingen *et al.*, 1980a,b). The difference in sensitivity should also be taken into consideration when discussing the fact that, after glucose administration to starved rats, liver glycogen is synthesized mainly from gluconeogenic precursors (Newgard *et al.*, 1983, 1984).

What are the biochemical mechanisms responsible for the difference in sensitivity? Glycogen is known to inhibit synthase phosphatase and may therefore exert a feedback inhibition on its own synthesis (De Wulf & Hers, 1968a; Watts & Malthus, 1980). To explain the difference in glucose sensitivity for [fructose 2,6-bisphosphate], several factors should be taken into consideration. First, the activity of phosphofructokinase-2 is lower in livers of fasted than of fed mice. Second, [fructose 6-phosphate] is lower in livers of fasted ($27 \pm 7 \text{ nmol/g}$; $n = 6$) than of fed ($57 \pm 12 \text{ nmol/g}$; $n = 8$) mice. Moreover, part of this fructose 6-phosphate could be bound to proteins (e.g. phosphoglucose isomerase), and the actual free concentration could be so low as to reach a critical value at which the inhibition of fructose 2,6-bisphosphatase by fructose 6-phosphate (K_i below $1 \mu\text{M}$, according to Van Schaftingen *et al.*, 1982a) could be, at least partially, relieved. Therefore, the activity of fructose 2,6-bisphosphatase could be greater than that of phosphofructokinase-2, and a cycling would occur between fructose 6-phosphate and fructose 2,6-bisphosphate with no net synthesis of fructose 2,6-bisphosphate, despite the presence of fructose 6-phosphate.

Regulation of fructose 2,6-bisphosphate metabolism in vivo by glucose

In isolated hepatocytes, the change in [fructose 6-phosphate] offers a likely explanation for the increase in [fructose 2,6-bisphosphate] brought about by glucose. *In vivo*, however, glucose does not increase [hexose 6-phosphates], nor does it alter the proportion of phosphofructokinase-2 in the active form. Moreover, [*sn*-glycerol 3-phosphate] and [phosphoenolpyruvate], which are known inhibitors of phosphofructokinase-2 (Van Schaftingen & Hers, 1981; Claus *et al.*, 1982), are increased after glucose administration (Hue, 1982a). Therefore, we can only speculate that the increase in [fructose 2,6-bisphosphate] observed after glucose injection *in vivo* may result either from a change in the activity of fructose 2,6-bisphosphatase or from changes in the concentration of some hitherto unknown effector(s) of phosphofructokinase-2/fructose 2,6-bisphosphatase.

Glucose administration to isolated rat hepatocytes, perfused rat livers or anaesthetized mice (Buschiazzo *et al.*, 1970; Bontemps *et al.*, 1978; Katz *et al.*, 1978; Nordlie *et al.*, 1980; Hue *et al.*, 1981; the present paper) causes an increase in [hexose 6-phosphate], whereas it has exactly the opposite effect in non-anaesthetized fed rats or mice (De Wulf & Hers, 1967; Hue & Hers, 1974; the present paper). The decrease observed *in vivo* has been interpreted as a result of the activation of glycogen synthase (Hers, 1976, 1981). This explanation is not entirely valid, since our results show that such a decrease occurs at low [glucose], with no activation of glycogen synthase. Other glucose-dependent mechanisms, such as a stimulation of glycolysis or an inhibition of glycogen breakdown, should be considered (for a discussion, see also Hue, 1982b). Although it is not possible to rule out a stimulation of glycolysis, an inactivation of phosphorylase is probably of minor importance. In non-anaesthetized mice or rats, phosphorylase is artifactually activated even after glucose administration probably because of adrenergic reactions (Stalmans *et al.*, 1974). This should lead to a stimulation of glycogen breakdown, yet [hexose 6-phosphates] are low. In contrast, in anaesthetized animals, phosphorylase activity remains low, but [hexose 6-phosphates] are increased. It is therefore difficult to explain the difference in [hexose 6-phosphates] between anaesthetized and non-anaesthetized animals only in terms of adrenaline and phosphorylase activation. Other processes, such as the pentose phosphate pathway or glucose 6-phosphatase activity, might also be stimulated by glucose. These glucose effects would be present only in conscious animals, inhibited by anaesthesia, and absent in isolated liver preparations.

We are grateful to Dr. M. H. Rider for his critical reading, to Mrs. L. Maisin for her technical assistance, and to Mrs. Th. Lambert for her skilful typing of the manuscript. L. H. is Maître de Recherches du Fonds National de la Recherche Scientifique.

References

- Bartrons, R., Hue, L., Van Schaftingen, E. & Hers, H. G. (1983) *Biochem. J.* **214**, 829–837
- Bontemps, F., Hue, L. & Hers, H. G. (1978) *Biochem. J.* **174**, 603–611
- Brunengraber, H., Boutry, M. & Lowenstein, J. M. (1973) *J. Biol. Chem.* **248**, 2656–2669
- Buschiazzo, H., Exton, J. H. & Park, C. R. (1970) *Proc. Natl. Acad. Sci. U.S.A.* **65**, 383–387
- Clark, D. G., Roghstad, R. & Katz, J. (1974) *J. Biol. Chem.* **249**, 2028–2036
- Claus, T. H., Schlumpf, J. R., El-Maghrabi, M. R. & Pilkis, S. J. (1982) *J. Biol. Chem.* **257**, 7541–7548
- De Wulf, H. & Hers, H. G. (1967) *Eur. J. Biochem.* **2**, 50–60
- De Wulf, H. & Hers, H. G. (1968a) *Eur. J. Biochem.* **6**, 552–557
- De Wulf, H. & Hers, H. G. (1968b) *Eur. J. Biochem.* **6**, 558–564
- Hers, H. G. (1976) *Annu. Rev. Biochem.* **45**, 167–189
- Hers, H. G. (1981) in *Short-term Regulation of Liver Metabolism* (Hue, L. & van de Werve, G., eds.), pp. 105–117, Elsevier/North-Holland, Amsterdam and New York
- Hers, H. G. & Van Schaftingen, E. (1982) *Biochem. J.* **206**, 1–12
- Hohorst, H. J. (1963a) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 134–138, Academic Press, New York and London
- Hohorst, H. J. (1963b) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 266–270, Academic Press, New York and London
- Hue, L. (1981) *Adv. Enzymol.* **52**, 247–331
- Hue, L. (1982a) *Biochem. J.* **206**, 359–365
- Hue, L. (1982b) in *Metabolic Compartmentation* (Sies, H., ed.), pp. 71–97, Academic Press, London and New York
- Hue, L. & Bartrons, R. (1984) *Biochem. J.* **192**, 263–271
- Hue, L. & Hers, H. G. (1974) *Biochem. Biophys. Res. Commun.* **58**, 532–539
- Hue, L., Bontemps, F. & Hers, H. G. (1975) *Biochem. J.* **152**, 105–114
- Hue, L., Feliu, J. E. & Hers, H. G. (1978) *Biochem. J.* **176**, 791–797
- Hue, L., Blackmore, P. F. & Exton, J. H. (1981) *J. Biol. Chem.* **256**, 8900–8903
- Katz, J. & Rognstad, R. (1976) *Curr. Top. Cell. Regul.* **10**, 237–289
- Katz, J., Wals, P. A., Golden, S. & Rognstad, R. (1975) *Eur. J. Biochem.* **60**, 91–101
- Katz, J., Wals, P. A. & Rognstad, R. (1978) *J. Biol. Chem.* **253**, 4530–4536
- Kitajima, S. & Uyeda, K. (1983) *J. Biol. Chem.* **258**, 7352–7357
- McGrane, M. M., El-Maghrabi, M. R. & Pilkis, S. J. (1983) *J. Biol. Chem.* **258**, 10445–10454

- Newgard, C. B., Hirsch, L. J., Foster, D. W. & McGarry, J. D. (1983) *J. Biol. Chem.* **258**, 8046–8052
- Newgard, C. B., Moore, S. V., Foster, D. W. & McGarry, J. D. (1984) *J. Biol. Chem.* **259**, 6958–6963
- Nordlie, R. C., Sukalski, K. A. & Alvares, F. L. (1980) *J. Biol. Chem.* **255**, 1834–1838
- Pilkis, S. J., El-Maghrabi, M. R., Pilkis, J. & Claus, T. H. (1981) *J. Biol. Chem.* **256**, 3619–3622
- Reinhart, G. D. & Lardy, H. A. (1980) *Biochemistry*, **19**, 1477–1484
- Riou, J. P., Claus, T. H., Flockhart, D. A., Corbin, J. D. & Pilkis, S. J. (1977) *Proc. Natl. Acad. Sci. U.S.A.* **74**, 4615–4619
- Sakakibara, R., Kitajima, S. & Uyeda, K. (1984) *J. Biol. Chem.* **259**, 41–46
- Seglen, P. O. (1974) *Biochim. Biophys. Acta* **338**, 317–336
- Stalmans, W. (1976) *Curr. Top. Cell. Regul.* **11**, 51–97
- Stalmans, W., De Wulf, H., Hue, L. & Hers, H. G. (1974) *Eur. J. Biochem.* **41**, 127–134
- Van Schaftingen, E. & Hers, H. G. (1981) *Biochem. Biophys. Res. Commun.* **101**, 1078–1084
- Van Schaftingen, E., Hue, L. & Hers, H. G. (1980a) *Biochem. J.* **192**, 263–271
- Van Schaftingen, E., Hue, L. & Hers, H. G. (1980b) *Biochem. J.* **192**, 887–895
- Van Schaftingen, E., Jett, M. F., Hue, L. & Hers, H. G. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 3483–3486
- Van Schaftingen, E., Davies, E. & Hers, H. G. (1982a) *Eur. J. Biochem.* **129**, 143–149
- Van Schaftingen, E., Lederer, B., Bartrons, R. & Hers, H. G. (1982b) *Eur. J. Biochem.* **129**, 191–195
- Watts, C. & Malthus, R. S. (1980) *Eur. J. Biochem.* **108**, 73–77
- Wollenberger, A., Ristau, O. & Schoffa, G. (1960) *Pflügers Arch. Gesamte Physiol. Menschen Tiere* **270**, 399–412
- Woods, H. F. & Krebs, H. A. (1971) *Biochem. J.* **125**, 129–139