Development of gluconeogenesis from dihydroxyacetone in rat hepatocytes during a feeding cycle and starvation

Belkacem AZZOUT and Jean PERET*

Centre de Recherches sur la Nutrition, CNRS, 9 rue Jules Hetzel, 92190 Meudon-Bellevue, France

(Received 24 October 1983/Accepted 6 December 1983)

Pyruvate kinase activity and the rates of gluconeogenesis and glycolysis in rat hepatocytes were evaluated by production of glucose and lactate + pyruvate from dihydroxyacetone during a feeding cycle or progressive starvation. In fed rats, during daylight (low food intake) and until darkness, gluconeogenesis progressively increased and glycolysis decreased slightly, but gluconeogenesis never exceeded glycolysis. During nocturnal feeding, gluconeogenesis and glycolysis returned to their morning rates. After 8 h starvation, an equal proportion of dihydroxyacetone was converted into glucose and into lactate + pyruvate. When glycogen was depleted (11 h of starvation), gluconeogenesis was maximal and glycolysis minimal. In fed and starved rats, the concentration of fructose 1,6-bisphosphate was the same. The activity ratio of pyruvate kinase (ratio of velocity at 0.5 mM-phosphoenolpyruvate to the maximum catalytic activity obtained with 4 mM-phosphoenolpyruvate) was high in crude extracts of cells incubated with dihydroxyacetone and low in $(\text{NH}_4)_2\text{SO}_4$ -treated extracts, but remained unchanged during the whole experiment. There was no correlation between the rates of gluconeogenesis and glycolysis from dihydroxyacetone and the activity ratio of pyruvate kinase.

It is known that hepatic gluconeogenesis in the rat is controlled at the level of the pyruvate/phosphoenolpyruvate cycle, and it has been suggested that inhibition of pyruvate kinase is a key mechanism in the activation of gluconeogenesis during starvation or after glucagon injections (reviewed by Exton, 1972). Indeed, hepatic L pyruvate kinase can be inactivated *in vitro* by a cyclic AMP-dependent phosphorylation (Ljungström *et al.*, 1974; Titanji *et al.*, 1976), and changes in the kinetic properties of the enzyme have been described after administration of glucagon *in vivo* or in hepatocytes incubated with glucagon and cyclic AMP (reviewed by Claus & Pilkis, 1981). Glucagon increases the phosphorylation of the enzyme (Riou *et al.*, 1978) and insulin prevents the inhibition induced by submaximal concentration of glucagon (Blair *et al.*, 1976; Feliu *et al.*, 1976; Claus *et al.*, 1979). Pyruvate kinase activity can also be modulated by changes in the concentrations of various allosteric effectors, in particular fructose 1,6-bisphosphate (Riou *et al.*, 1976; Van Berkel *et al.*, 1977; Claus *et al.*, 1979).

The flux through pyruvate kinase can be

estimated in isolated hepatocytes by measuring the rate or production of lactate and pyruvate from dihydroxyacetone (Pilkis *et al.*, 1976). Dihydroxyacetone is first converted into dihydroxyacetone phosphate by glycerol kinase and then into either glucose or lactate + pyruvate. The flux through pyruvate kinase is depressed by glucagon or starvation, while the rate of gluconeogenesis is increased concomitantly (Pilkis *et al.*, 1976; Foster & Blair, 1978; Claus *et al.*, 1979).

The above observations were based only on studies using hepatocytes from fed or 24–72 h-starved animals, incubated with or without glucagon. However, it appears that both starvation and glucagon treatment resulted in a maximal increase in the rate of gluconeogenesis and a maximal decrease in glycolysis and pyruvate kinase activity. One can wonder what is the duration of starvation required to bring about the full activation of gluconeogenesis and inactivation of glycolysis, and what type of kinetics are displayed during the induction or inhibition of these processes. In an attempt to answer these questions, we have investigated the time sequence of the effects of starvation and high-carbohydrate feeding on the rates of production of glucose and lactate + pyru-

* To whom reprint requests should be addressed.

vate from dihydroxyacetone in isolated rat hepatocytes and on the activity of pyruvate kinase in the same cells.

Materials and methods

Chemicals

Substrates, coenzymes and enzymes were supplied by Boehringer (Meylan, France). Other chemicals were purchased from Merck (Coger, Paris, France).

Animals and experimental design

Male Wistar rats (Janvier, Le Genest, France) weighing 260–300 g were used for the preparation of isolated hepatocytes. They were housed under controlled conditions providing light from 07:00 to 19:00 h and were adapted to a high-carbohydrate diet (70% carbohydrate, 10% protein, 8% lipid; Peret *et al.*, 1981). The rats were then divided into two groups: the first had free access to the high-carbohydrate diet, whereas the second group was starved. For convenience, animals will be respectively referred to as 'fed' and 'starved'. Four animals in each group were killed every 3 h over 24 h in the fed group and over 48 h in the starved group.

Isolation of hepatocytes

Hepatocytes were prepared by the method of Berry & Friend (1969) as modified by Seglen (1976). The final cell pellet was resuspended in 35–40 ml of Krebs–Henseleit (1932) bicarbonate buffer (pH 7.4) and the viability was routinely checked by staining with Trypan Blue. Only preparations in which viability exceeded 85% were used.

Assay of metabolite concentrations and measurement of rates of production of glucose and lactate + pyruvate from dihydroxyacetone

Hepatocytes (about 8×10^6 cells) were incubated at 37°C for 10 min in a final volume of 4 ml of Krebs–Henseleit buffer. The flasks were gassed with O₂/CO₂ (19:1), sealed, and the cell suspension was further incubated at 37°C for 10 min after the addition of dihydroxyacetone (5 mM final concn.) in a shaking water bath. Each incubation was performed in duplicate (with or without dihydroxyacetone). Incubations were terminated by the addition of 5 ml of ice-cold HClO₄ (40%, v/v). After neutralization, glucose (Huggett & Nixon, 1957), lactate (Gutmann & Wahlefeld, 1974), pyruvate (Czok & Lamprecht, 1974) and fructose 1,6-bisphosphate (Michal & Beutler, 1974) were assayed by standard enzymic methods. The rates of production of glucose and lactate + pyruvate from dihydroxyacetone were measured by

subtracting the amounts of glucose and lactate + pyruvate produced in the absence of dihydroxyacetone from the total amounts produced after addition of dihydroxyacetone. The concentration of metabolites in isolated hepatocytes and the rates of production of glucose and of lactate + pyruvate were expressed per 10⁶ hepatocytes.

Measurement of pyruvate kinase activity

After incubation, the hepatocytes (20×10^6 – 25×10^6 cells) were rapidly (15–20 s) sedimented, the supernatant was removed by aspiration, and the cell pellet was frozen in liquid N₂. The cells were homogenized with an Ultra-Turrax homogenizer in ice-cold Krebs–Henseleit buffer containing 1 mM-mercaptoethanol (Van Berkel *et al.*, 1977). The homogenates were centrifuged for 10 min at 50 000 g at 0°C. The activity of pyruvate kinase was immediately determined in the crude supernatant. In some experiments, samples of the supernatant fraction were treated with cold saturated (NH₄)₂SO₄ to give a final concentration of 60% (Foster & Blair, 1978; Claus *et al.*, 1979) to precipitate pyruvate kinase and to eliminate fructose 1,6-bisphosphate. The precipitates were desalted on a Sephadex G-25 (medium grade) column before the determination of enzyme activity. Pyruvate kinase activity in the crude supernatants and in (NH₄)₂SO₄-treated and desalted fractions was measured as described by Blair *et al.* (1976). The reaction was initiated by adding various concentrations (0.1–6 mM) of phosphoenolpyruvate and monitoring the oxidation of NADH at 340 nm at 37°C. The maximum velocity ($V_{\max}$) was measured at 4 mM-phosphoenolpyruvate and was similar to that measured at 0.5 mM-phosphoenolpyruvate and in the presence of 0.5 mM-fructose 1,6-bisphosphate. The results were expressed as $V_{\max}$ or as the ratio of velocity at 0.5 mM-phosphoenolpyruvate to the maximum catalytic activity obtained with 4 mM-phosphoenolpyruvate [$(V_{0.5}/V_{\max}) \times 100$]. This ratio was termed the activity ratio of pyruvate kinase (Blair *et al.*, 1976). Protein was measured as described by Lowry *et al.* (1951), with bovine serum albumin as standard; 10⁶ cells usually yielded 1.0–1.3 mg of soluble protein. Specific activity of the enzyme was expressed as nmol of substrate transformed/min per mg of protein at 37°C.

Glycogen was measured in the frozen cell pellet by the method of Roehrig & Allred (1974).

Expression of results

The results were expressed as means $\pm$ S.E.M. Statistical analyses (analysis of variance, correlation) were performed as described by Snedecor & Cochran (1967).

Results

Rates of gluconeogenesis and glycolysis from dihydroxyacetone

In fed rats, the rate of gluconeogenesis from dihydroxyacetone was much lower than that observed in starved animals (Fig. 1a). Nevertheless, gluconeogenesis was 2.5 times higher ($P < 0.05$) during the period of lower food intake (07:00–19:00h) (35 nmol of glucose/10 min per 10^6 cells) than during the period of nocturnal feeding (19:00–07:00h) (14 nmol of glucose/10 min per 10^6 cells). In starved rats, the rate of gluconeogenesis increased 5 h after the beginning of starvation, and reached a peak after 11–20 h (around 140 nmol of glucose/10 min per 10^6 cells) at a value 5–7-fold higher than in fed animals. Thereafter, glucose production from dihydroxyacetone remained at a plateau until the end of the experiment (48 h).

In both fed and starved rats, the rate of production of lactate + pyruvate from dihydroxyacetone exhibited similar variations during the first light period (07:00–19:00h) (Fig. 1b). It was

high during the first hours (around 200 nmol of lactate + pyruvate/10 min per 10^6 cells) and fell just before the onset of darkness. In fed rats it rose again 3 h after onset of darkness and remained elevated during the period of nocturnal food intake. In starved rats, the rate of production of lactate + pyruvate decreased progressively and amounted to only 30% of the fed value after 48 h of starvation.

Relationship between gluconeogenesis and glycolysis

Dihydroxyacetone enters the glycolytic pathway at the level of triose phosphates and is converted into glucose or pyruvate + lactate depending on the metabolic state (Pilkis *et al.*, 1976). Thus, for a better understanding of the time-related changes between the two fluxes during feeding and starvation, we have expressed production of glucose and lactate + pyruvate from dihydroxyacetone as percentage of total dihydroxyacetone used [$2 \times (\text{nmol of glucose produced/10 min per } 10^6 \text{ cells}) + (\text{nmol of lactate + pyruvate produced/10 min per } 10^6 \text{ cells})$].

Fig. 1. Rates of production of glucose (a) and lactate + pyruvate (b) from dihydroxyacetone in isolated hepatocytes from fed or starved rats

Isolated hepatocytes were prepared from rats having free access to a high-carbohydrate diet for 24 h (●) and from rats starved over 48 h (▲). Hepatocytes were incubated with 5 mM-dihydroxyacetone for 10 min. The rates of production of glucose and lactate + pyruvate from dihydroxyacetone were measured. Results plotted are means $\pm$ S.E.M. (bars) for four different animal cell preparations.

Fig. 2. Percentage of total dihydroxyacetone used converted into glucose or into lactate + pyruvate in isolated hepatocytes from fed or starved rats

Isolated hepatocytes from fed (●, ○) and starved (▲, △) rats were incubated with 5 mM-dihydroxyacetone, and the rates of production of glucose and lactate + pyruvate were calculated. The percentage of total dihydroxyacetone (DHA) used $[2 \times (\text{glucose produced}/10 \text{ min per } 10^6 \text{ cells}) + (\text{lactate} + \text{pyruvate produced}/10 \text{ min per } 10^6 \text{ cells})]$ converted into glucose (●, ▲) or into lactate + pyruvate (○, △) was calculated by using the following formula:

$$\frac{\text{glucose or lactate + pyruvate produced}}{\text{total dihydroxyacetone used}} \times 100$$

In fed rats, the proportion of dihydroxyacetone converted into glucose was low and exhibited no marked diurnal variations (Fig. 2a). Nevertheless, the conversion of dihydroxyacetone into glucose was higher during the light period (20–30%), when hepatocyte glycogen was decreased (Fig. 3) than during the period of nocturnal feeding (10%), when glycogen concentrations in hepatocytes were high (Fig. 3). This shows that, although gluconeogenesis is indeed stimulated to some extent during the period of lower food intake (07:00–19:00h) it is quantitatively of minor importance compared with a strongly predominant glycolysis.

In starved animals the proportion of dihydroxyacetone converted into glucose increased as starvation progressed (Fig. 2b). After starvation for 8 h, 50% of dihydroxyacetone was converted into glucose. After 11 h, when hepatocyte glycogen con-

centration was lowest (Fig. 3), gluconeogenesis became predominant and 70–75% of dihydroxyacetone was converted into glucose.

Changes in the activity of pyruvate kinase

Maximum pyruvate kinase activity. As reported previously (Foster & Blair, 1978; Claus *et al.*, 1979), the maximum activity of pyruvate kinase was not altered by addition of dihydroxyacetone to isolated hepatocytes or by removal of fructose 1,6-bisphosphate. Therefore the changes in maximum activity of pyruvate kinase are reported only for cells incubated with dihydroxyacetone (Fig. 4a). In fed rats, pyruvate kinase activity underwent little diurnal variation. However, the highest activity of the enzyme was observed during the feeding period (19:00–07:00h), as previously reported (Peret *et al.*, 1981). During the first 17 h of starvation,

Fig. 3. Glycogen concentration in isolated hepatocytes from fed and starved rats. Isolated hepatocytes from fed (●) or starved (▲) rats were incubated for 10 min with 5 mM-dihydroxyacetone. Results shown are means for four different cell preparations, and the bars indicate the S.E.M.

Fig. 4. Variations in the catalytic activity of pyruvate kinase (a, b, c) and fructose 1,6-bisphosphate concentrations (d) in isolated hepatocytes from fed and starved rats.

Isolated hepatocytes prepared from fed (●) and starved (▲) rats were incubated with 5 mM-dihydroxyacetone for 10 min. Pyruvate kinase activity was assayed in crude extracts or after precipitation with cold saturated $(\text{NH}_4)_2\text{SO}_4$ to give a final concentration of 60%. The results were expressed as maximal activity of pyruvate kinase (V_{max}) measured at 4 mM-phosphoenolpyruvate (a) and as activity ratio of pyruvate kinase $[(V_{0.5}/V_{\text{max}}) \times 100]$ (b, c). Fructose 1,6-bisphosphate (d) was measured in neutralized perchlorate extracts. Results shown are means for four different cell preparations, and the bars indicate S.E.M.

pyruvate kinase activity followed the same pattern as in fed rats. After 20 h of starvation, enzyme activity fell by 25%, and reached a plateau around 60% of the fed value for the last 12 h of starvation. As described previously (Krebs & Eggleston, 1965; Van Berkel *et al.*, 1977; Kohl & Cottam, 1977; Hopkirk & Bloxham, 1979), a decrease in total pyruvate kinase in liver homogenates occurred during starvation, and this resulted from a de-

crease in pyruvate kinase synthesis (Cladaras & Cottam, 1980). However, during early starvation the onset of gluconeogenesis (see Figs. 1 and 2) was not accompanied by a decrease in total pyruvate kinase activity. We therefore decided to investigate whether the rates of gluconeogenesis and of glycolysis from dihydroxyacetone could be related to the activity ratio of pyruvate kinase during feeding and starvation.

Activity ratio of pyruvate kinase. In both fed and starved rats, the activity ratio of pyruvate kinase was high in crude extracts of cells incubated with dihydroxyacetone (Fig. 4b) and low in $(\text{NH}_4)_2\text{SO}_4$ -treated extracts (Fig. 4c), but remained unchanged during the whole experimental period.

As shown by others (Claus *et al.*, 1979), addition of dihydroxyacetone increased fructose 1,6-bisphosphate 2–5-fold, from 0.40 and 0.12 nmol/10⁶ cells respectively in fed and 48h-starved rats (results not shown) to concentrations between 0.90 and 1.00 nmol/10⁶ cells in both groups at all experimental times (Fig. 4d).

There was no correlation between the rates of gluconeogenesis and glycolysis from dihydroxyacetone (Figs. 1a, 1b, 2a and 2b) and the activity ratio of pyruvate kinase measured in crude extracts or in $(\text{NH}_4)_2\text{SO}_4$ -treated extracts from cells incubated with dihydroxyacetone (Figs. 4b and 4c).

Discussion

The present data (Figs. 1 and 2) show that the rates of production of glucose and lactate + pyruvate by isolated hepatocytes incubated with dihydroxyacetone change progressively in opposite directions during a feeding cycle or a period of starvation, and that these changes are closely related with the modifications of hepatic [glycogen]. However, no change was found in the activity ratio of pyruvate kinase, nor in [fructose 1,6-bisphosphate] in hepatocytes incubated with dihydroxyacetone (Figs. 4b and 4d). This suggests that another factor must be involved in the control of glycolysis and gluconeogenesis during feeding or starvation in hepatocytes incubated with dihydroxyacetone. In the absence of added hormones, in hepatocytes this factor could be the availability of substrates and/or effectors.

Dihydroxyacetone enters the glycolytic pathways at the level of triose phosphates after its conversion into dihydroxyacetone phosphate by D-triokinase and/or glycerol kinase (reviewed by Veneziale, 1976). Its subsequent partition between glucose or pyruvate + lactate depends on the metabolic status of the rat: starved (gluconeogenesis) or fed (glycolysis) (Pilkis *et al.*, 1976; Foster & Blair, 1978). Moreover, gluconeogenesis from dihydroxyacetone is independent of the rate of resynthesis of phosphoenolpyruvate from pyruvate (Veneziale, 1971; Blair *et al.*, 1973; Pilkis *et al.*, 1976). Fructose 2,6-bisphosphate, which controls the fructose 6-phosphate/fructose 1,6-bisphosphate cycle in liver (reviewed by Hers & Van Schaftingen, 1982), could be involved in the switch-on from glycolysis to gluconeogenesis during starvation in hepatocytes incubated with dihydroxyacetone. Although [fructose 2,6-bisphos-

phate] is decreased in rat hepatocytes and livers after 24h of starvation (Richards *et al.*, 1981; Pilkis *et al.*, 1982) when glycogen is also exhausted, it can be brought back within the normal range by addition of glucose to the hepatocytes (Van Schaftingen *et al.*, 1980). Furthermore, fructose 1,6-bisphosphate has no effect on 6-phosphofructo-2-kinase and fructose 2,6-bisphosphate activities (Pilkis *et al.*, 1982).

Previous studies (Pilkis *et al.*, 1976; Foster & Blair, 1978) have shown that the glucagon-induced increase in gluconeogenesis and the concurrent decrease in glycolysis in hepatocytes from fed or starved rats incubated with dihydroxyacetone was parallel with a decrease in the activity ratio of pyruvate kinase and a decrease in [fructose 1,6-bisphosphate] in hepatocytes. Moreover, removal of fructose 1,6-bisphosphate from the extracts did not alter the glucagon-induced low affinity of pyruvate kinase for phosphoenolpyruvate (Claus *et al.*, 1979). Starvation induces a rise in plasma glucagon and hepatic cyclic AMP concentrations, especially after 11h of starvation (Seitz *et al.*, 1977), but our results show a similar and low activity ratio of pyruvate kinase in $(\text{NH}_4)_2\text{SO}_4$ -treated extracts from fed and starved rats. This suggests that the effect of glucagon on the phosphorylation of pyruvate kinase which exists *in vitro* might be lost during the preparation of hepatocytes. In keeping with this, it has been shown that the activity ratio of pyruvate kinase in crude extracts of perfused liver, or isolated hepatocytes from fed or starved rats, was always high and could be decreased only by addition of glucagon or cyclic AMP (Blair *et al.*, 1976; Claus *et al.*, 1979).

In summary, the present results show that the rates of gluconeogenesis and glycolysis from dihydroxyacetone change progressively in opposite directions during a feeding cycle or during starvation, but that there is no correlation between these changes and the activity ratio of pyruvate kinase. [Fructose 2,6-bisphosphate] could be involved in the switch-on of gluconeogenesis and glycolysis from dihydroxyacetone during starvation and the feeding cycle.

We are indebted to Dr. Jean-Paul Riou and Dr. Jean Girard for helpful discussion during the course of this work. We thank S. Seige for the careful preparation of the manuscript.

References

- Berry, M. N. & Friend, D. S. (1969) *J. Cell Biol.* **43**, 506–520
- Blair, J. B., Cook, D. E. & Lardy, H. A. (1973) *J. Biol. Chem.* **248**, 3601–3607.

- Blair, J. B., Cimbala, M. A., Foster, J. L. & Morgan, R. L. (1976) *J. Biol. Chem.* **251**, 3756–3762
- Cladaras, C. & Cottam, G. L. (1980) *Arch. Biochem. Biophys.* **200**, 426–433
- Claus, T. H. & Pilkis, S. J. (1981) *Biochem. Actions Horm.* **8**, 209–271
- Claus, T. H., El-Maghrabi, M. R. & Pilkis, S. J. (1979) *J. Biol. Chem.* **254**, 7855–7864
- Czok, R. & Lamprecht, W. (1974) in *Methods of Enzymatic Analysis*, 2nd edn. (Bergmeyer, H. U., ed.), vol. 3, pp. 1446–1451, Academic Press, New York
- Exton, J. H. (1972) *Metab. Clin. Exp.* **21**, 945–990
- Feliu, J. E., Hue, L. & Hers, H. G. (1976) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 2762–2766
- Foster, J. L. & Blair, J. B. (1978) *Arch. Biochem. Biophys.* **189**, 263–276
- Gutmann, I. & Wahlefeld, A. W. (1974) in *Methods of Enzymatic Analysis*, 2nd edn. (Bergmeyer, H. U., ed.), vol. 3, pp. 1464–1468, Academic Press, New York
- Hers, H. G. & Van Schaftingen, E. (1982) *Biochem. J.* **206**, 1–12
- Hopkirk, T. J. & Bloxham, D. P. (1979) *Biochem. J.* **182**, 383–397
- Huggett, A. St. G. & Nixon, D. A. (1957) *Biochem. J.* **66**, 12P
- Kohl, E. A. & Cottam, G. L. (1977) *Biochim. Biophys. Acta.* **484**, 49–58
- Krebs, H. A. & Henseleit, K. (1932) *Hoppe-Seyler's Z. Physiol. Chem.* **210**, 33–36
- Krebs, H. A. & Eggleston, L. V. (1965) *Biochem. J.* **94**, 3C–4C
- Ljungström, O., Helmquist, G. & Engström, L. (1974) *Biochim. Biophys. Acta* **358**, 289–298
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Michal, G. & Beutler, H.-O. (1974) in *Methods of Enzymatic Analysis*, 2nd edn. (Bergmeyer, H. U., ed.), vol. 3, pp. 1314–1319, Academic Press, New York
- Peret, J., Foustock, S., Chanez, M., Bois-Joyeux, B. & Assan, R. (1981) *J. Nutr.* **111**, 1173–1184
- Pilkis, S. J., Riou, J. P. & Claus, T. H. (1976) *J. Biol. Chem.* **251**, 7841–7852
- Pilkis, S. J., Chrisman, T., Burgess, B., McGrane, M., Colosia, A., Pilkis, J., Claus, T. H. & El-Maghrabi, M. R. (1982) *Adv. Enzyme Regul.* **21**, 147–182
- Richards, C. S., Furuya, E. S. & Uyeda, K. (1981) *Biochem. Biophys. Res. Commun.* **100**, 1673–1679
- Riou, J. P., Claus, T. H. & Pilkis, S. J. (1976) *Biochem. Biophys. Res. Commun.* **73**, 591–599
- Riou, J. P., Claus, T. H. & Pilkis, S. J. (1978) *J. Biol. Chem.* **253**, 656–659
- Roehrig, K. L. & Allred, J. B. (1974) *Anal. Biochem.* **58**, 414–421
- Seglen, P. O. (1976) *Methods Cell Biol.* **13**, 29–83
- Seitz, E. A., Müller, M. J., Krone, W. & Tarnowski, W. (1977) *Arch. Biochem. Biophys.* **183**, 647–663
- Snedecor, G. W. & Cochran, W. G. (1967) *Statistical Methods*, 6th edn., Iowa State University Press, Ames, IA
- Titanji, V. P. K., Zetterqvist, O. & Engström, L. (1976) *Biochim. Biophys. Acta* **422**, 98–108
- Van Berkel, T. J. C., Kruijt, J. & Koster, J. (1977) *Eur. J. Biochem.* **81**, 423–432
- Van Schaftingen, E., Hue, L. & Hers, H. G. (1980) *Biochem. J.* **192**, 887–895
- Veneziale, C. M. (1971) *Biochemistry* **10**, 3443–3447
- Veneziale, C. M. (1976) in *Gluconeogenesis: its Regulation in Mammalian Species* (Hanson, R. W. & Mehlerman, M. A., eds.), pp. 463–480, John Wiley and Sons, New York