Regulation of renal and hepatic pyruvate dehydrogenase complex on carbohydrate re-feeding after starvation

Possible mechanisms and a regulatory role for thyroid hormone

Mark J. HOLNESS and Mary C. SUGDEN*

Department of Chemical Pathology, London Hospital Medical College, Turner Street, London E1 2AD, U.K.

The work investigated the mechanisms for modulation of renal and hepatic pyruvate dehydrogenase complex (PDH) activities after carbohydrate re-feeding of 48 h-starved rats, and identified a regulatory role for tri-iodothyronine. Glucose re-feeding decreased blood concentrations of lipid fuels in both euthyroid and hyperthyroid rats. This treatment was not associated with re-activation of hepatic PDH in either group of rats, or of renal PDH in hyperthyroid rats (where activity was already high), but it increased renal PDH in euthyroid rats. Dichloroacetate (DCA), an activator of PDH kinase, increased renal PDH activities in euthyroid rats, but not hyperthyroid rats, and effects of glucose re-feeding or hyperthyroidism were no longer apparent. These treatments therefore exert their effects on renal PDH through changes in PDH kinase. DCA re-activation of hepatic PDH was more marked in hyperthyroid than in euthyroid rats, suggesting that, under conditions of inhibited kinase activity, PDH phosphatase is more active in livers of hyperthyroid rats. The limited effect of DCA on hepatic PDH in euthyroid rats was potentiated by glucose re-feeding or insulin, but not by inhibition of lipolysis, demonstrating a direct effect of insulin to increase hepatic PDH phosphatase. Glucose re-feeding, inhibition of lipolysis or insulin administration did not increase hepatic PDH in DCA-treated hyperthyroid rats, indicating that effects of hyperthyroidism and of insulin on PDH phosphatase are not additive.

INTRODUCTION

Two short-term mechanisms for restricting the activity of the pyruvate dehydrogenase complex (PDH; EC 1.2.4.1+2.3.1.12+1.8.1.4) have been recognized to operate in starvation (reviewed in [1] and [2]). First, the activity of the complex becomes progressively less as the intramitochondrial [acetyl-CoA]/[CoA] and [NADH]/[NAD⁺] ratios are increased. This occurs through end-product inhibition as a consequence of increased oxidation of lipid fuels. Second, complex activity is inhibited by reversible phosphorylation of its α -subunit catalysed by PDH kinase (EC 2.7.1.99). Kinase activity is increased by increasing mitochondrial [ATP]/[ADP], [acetyl-CoA]/[CoA] and [NADH]/[NAD⁺] ratios, and so phosphorylation is promoted under conditions where end-product inhibition occurs. During prolonged starvation, further mechanisms of control may operate. In heart [3] and liver [4], phosphorylation is accelerated by increased biosynthesis of PDH kinase or of a protein activator of the kinase, and in heart (and possibly other tissues), multi-site phosphorylation of PDH retards re-activation by dephosphorylation catalysed by PDH phosphatase (EC 3.1.3.43) [5,6].

Hepatic PDH in 48 h-starved rats is refractory to re-activation by decreased fat-fuel oxidation, as demonstrated by the finding that inhibition of mitochondrial fatty acid uptake by 2-tetradecylglycidate does not re-activate hepatic PDH in 48 h-starved rats [7]. Glucose re-feeding also fails to re-activate hepatic PDH in

48 h-starved rats [8], despite increased insulin concentrations (see [9]), increased blood glucose concentrations [8] and decreased concentrations of fatty acids and ketone bodies [8]. The physiological significance of retarded re-activation of hepatic PDH after re-feeding may be to ensure that hepatic carbon flux remains directed towards glucose 6-phosphate formation, thereby permitting utilization of lactate and pyruvate for glycogen synthesis via the gluconeogenic pathway ([8],[10]–[12], reviewed in [13]). In support of this, hepatic glycogen deposition after re-feeding is impaired under conditions where the proportion of hepatic PDH in the active form (PDH_a) is increased [8]. The presence of relatively low PDH activity in liver may also explain why glucose re-feeding fails to increase hepatic lipogenesis in starved rats [8,10,14], although increases are observed in fed animals [9,14,15]. The delayed re-activation of PDH in response to glucose is in marked contrast with the immediate re-activation observed in adipose tissue [14] and kidney [8]. The former tissue resembles liver in that it is an important site of lipogenesis. The latter tissue, like liver, is gluconeogenic in starvation, but differs from liver in its mode of regulation of PDH, partial re-activation being observed after administration of 2-tetradecylglycidate [7].

Nothing is yet known about the control of hepatic or renal PDH re-activation immediately after re-feeding of 48 h-starved rats, except for reversal of inhibition *in vivo* by 24–48 h of carbohydrate re-feeding. The present experiments were directed towards elucidating this

Abbreviations used: PDH, pyruvate dehydrogenase complex; PDH_a, its active form; T₃, tri-iodothyronine; DCA, dichloroacetate; NEFA, non-esterified fatty acids; MPCA, 5-methylpyrazole-3-carboxylic acid.

* To whom correspondence should be addressed.

problem. In addition we have chosen to study the effects of hyperthyroidism on the response of hepatic and renal PDH to starvation and re-feeding. Hyperthyroidism has been demonstrated not only to attenuate starvation-induced decreases in renal PDH activities [16], but also to permit increased hepatic lipogenesis after glucose re-feeding of starved rats [9,14,17]. Delineation of the metabolic effects of thyroid hormones on PDH activities in these tissues is, apart from its intrinsic interest, a means of assessing the relative importance of different factors in regulating PDH activity *in vivo*.

MATERIALS AND METHODS

Materials

Sources of materials were as in [8] and [16].

Treatment of rats

Female albino Wistar rats (180–220 g) were starved for 48 h (grid-bottomed cages). Rats were made hyperthyroid by the subcutaneous injection of T_3 (100 μ g/100 g body wt. per day) during the period of starvation as described previously [16].

Experiments were started on day 3 between 08:30 and 09:30 h, when glucose (2 mmol/100 g body wt.) or water (1 ml/100 g body wt.) was administered intragastrically under light diethyl ether anaesthesia (see [15] and [18]). In some experiments dichloroacetate (DCA) [0.25 ml of a 5% (w/v) solution in 0.15 M-NaCl, pH 7.4] or 0.15 M-NaCl was injected intraperitoneally at the time of intubation and at 0.5 h intervals thereafter until rats were sampled at 2.0 h (see [8]). 5-Methylpyrazole-3-carboxylic acid (MPCA; 1 mg/100 g body wt. in 0.15 M-NaCl) was injected intraperitoneally at 1 h before rats were killed in order to inhibit lipolysis (see [16]). Insulin (2 units) was injected subcutaneously at 0.5 h before sampling.

Enzyme and metabolite assays

PDH complex, active form (PDH_a), total PDH and citrate synthase (EC 4.1.3.7) activities were measured in

freeze-clamped tissue extracts as described in [7]. Further details are given in [19]. Serum was included to inhibit proteolysis. A unit of enzyme activity converts 1 μ mol of substrate into product/min at 30 °C. PDH results have been expressed relative to citrate synthase (see [7]) in order to correct for possible differences in extraction of mitochondria. T_3 treatment decreased renal total PDH activity by approx. 17% (see [16]), but, as reported by others [20] was without effect on hepatic total PDH activity. Citrate synthase activities were similar in control and T_3 -treated rats in kidney, although in liver there was a slight, but significant, increase in T_3 -treated rats (Table 1). Plasma obtained at the time of sampling was assayed for non-esterified fatty acids (NEFA), and concentrations of the ketone bodies (D-3-hydroxybutyrate and acetoacetate) and glucose were determined in KOH-neutralized $HClO_4$ extracts (see [8] for details of methods).

Statistics

Results are presented as means $\pm$ S.E.M. for the numbers of rats (n) specified. Statistical differences were tested by Student's unpaired t test.

RESULTS AND DISCUSSION

Renal and hepatic PDH activities after glucose administration to 48 h-starved, euthyroid or hyperthyroid rats

After re-feeding there was a progressive decline in blood NEFA and ketone-body concentrations, which was evident in both control and hyperthyroid rats (Fig. 1a). In euthyroid rats this was associated with immediate re-activation of renal, but not of hepatic, PDH (Fig. 1b) (see also [8]). There was a significant effect of hyperthyroidism to increase renal PDH activity in the starved state, but, because glucose administration did not significantly increase activity in hyperthyroid rats, differences in renal PDH activities between euthyroid

Fig. 1. Effects of glucose re-feeding on blood concentrations of lipid fuels and on renal and hepatic PDH activities in 48 h-starved, euthyroid or hyperthyroid rats

For experimental details see the text. Blood ketone-body ($\blacktriangle$, $\triangle$) and fatty acid ($\bullet$, $\circ$) concentrations after glucose re-feeding are shown in (a) and renal ($\bullet$, $\circ$) and hepatic ($\blacktriangle$, $\triangle$) PDH_a activities are shown in (b). Filled symbols represent euthyroid rats, and open symbols hyperthyroid rats. Error bars have been omitted for clarity. In (a), effects of glucose re-feeding to decrease ketone-body and fatty acid concentrations were statistically significant ($P < 0.01$) at all time points in both euthyroid and hyperthyroid rats, and therefore only significant effects of hyperthyroidism are indicated ($\dagger P < 0.05$). In (b), statistically significant effects of glucose re-feeding are indicated by: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; and those of hyperthyroidism by: $\dagger P < 0.05$; $\dagger\dagger P < 0.01$.

and hyperthyroid rats were no longer apparent after re-feeding (Fig. 1b). We have previously demonstrated that the administration of MPCA (an inhibitor of lipolysis [21]) to starved hyperthyroid rats does not increase renal PDH activity (cf. starved euthyroid rats) ([16]; see also Table 1). The present results confirm that the activity of renal PDH in hyperthyroid rats is insensitive to changes in fat-fuel availability.

Hyperthyroidism was without effect on either hepatic PDH_a activity in the starved state, or on the response of hepatic PDH to glucose re-feeding (Fig. 1b). Again, hepatic PDH re-activation was not observed, and, although an effect of hyperthyroidism to increase flux through hepatic PDH without a change in its activity state is not excluded, the results suggest that hyperthyroidism increases hepatic lipogenesis after glucose re-feeding at loci distal to PDH.

Effects of DCA and MPCA

The pyruvate analogue DCA activates tissue PDH through inhibition of PDH kinase [22]. As the proportion of active PDH complex depends on the activity ratio of PDH kinase to PDH phosphatase, DCA may be used to inhibit kinase activity sufficiently to be able to determine whether increases in PDH activities are mediated predominantly through changes in activity of kinase or phosphatase. This approach has been successfully utilized previously to demonstrate that changes in cardiac PDH_a in response to ischaemia result from changes in PDH kinase activities [23], and that changes in adipose-tissue PDH_a in response to insulin result from changes in PDH phosphatase [see 24–26].

DCA administration increased renal PDH activities in euthyroid rats, but not in hyperthyroid rats (Table 1). This indicates that PDH-phosphatase activity is the same in both hormonal states. Neither glucose re-feeding nor hyperthyroidism influenced renal PDH in DCA-treated rats. Because DCA acts only on PDH kinase, we conclude that both these treatments exert their effects on renal PDH through changes in PDH kinase activity.

As previously reported [8], DCA re-activation of hepatic PDH in euthyroid rats is increased by glucose re-feeding (Table 1). DCA re-activation of the complex was not, however, potentiated by MPCA, although, as after glucose administration [8], decreased hepatic ketone-body concentrations and [3-hydroxybutyrate]/[acetoacetate] ratios (indicative of a decreased [acetyl-CoA]/[CoA] and of a more oxidized mitochondrial redox state) were observed (results not shown). The results indicate that activation of hepatic PDH in response to glucose in DCA-treated euthyroid rats is not due to changes in rates of fat oxidation (see [8]) or exchange of pyruvate for acetoacetate [27], but is a consequence of direct or insulin-mediated effect of glucose (or its derivatives) on PDH phosphatase. This conclusion assumes that DCA inhibits PDH kinase such that its activity is negligible compared with that of PDH phosphatase. In contrast with its relatively limited effect on hepatic PDH in euthyroid rats, DCA caused marked activation of hepatic PDH in hyperthyroid rats (Table 1), and its effect was not potentiated by the administration of glucose or insulin. It can be concluded that, under conditions of decreased PDH kinase activity, PDH phosphatase is more active in liver of hyperthyroid rats. Further experiments are needed to determine whether this effect of hyperthyroidism is the result of decreased

Table 1. Effects of glucose, DCA and MPCA on renal and hepatic PDH_a in control and (T₃)-treated 48 h-starved rats

Tissue	Treatment	Citrate synthase		PDH complex (active form)	
		(units/g wet wt.)		(% of total complex)	
		Control	T ₃ -treated	Control	T ₃ -treated
Kidney	Water	21.1 ± 1.2 (7)	22.2 ± 0.9 (6)	17.8 ± 1.3 (7)	44.5 ± 2.2 (6)†††
	Glucose	20.0 ± 1.6 (10)	20.6 ± 1.2 (7)	36.6 ± 2.5 (10)***	44.6 ± 3.4 (7)
	DCA	20.7 ± 0.7 (9)	21.9 ± 0.5 (8)	36.1 ± 2.5 (9)†††	42.9 ± 5.2 (8)
	DCA + glucose	22.1 ± 0.7 (9)	18.6 ± 1.7 (6)	37.2 ± 2.2 (9)	46.3 ± 6.4 (6)
	MPCA	18.5 ± 0.3 (6)	22.0 ± 0.8 (6)	39.2 ± 3.2 (6)†††	42.6 ± 3.4 (6)
Liver	Water	9.9 ± 0.6 (11)	11.9 ± 1.0 (10)	0.9 ± 0.1 (11)	0.9 ± 0.1 (10)
	Glucose	9.7 ± 0.5 (4)	11.5 ± 0.5 (7)†	0.6 ± 0.2 (4)	0.7 ± 0.2 (7)
	DCA	9.2 ± 0.7 (9)	11.9 ± 0.6 (8)†	8.5 ± 1.2 (9)†††	33.2 ± 1.9 (8)†††††
	DCA + glucose	9.4 ± 0.5 (9)	11.6 ± 0.8 (6)†	24.7 ± 2.7 (9)†††	37.4 ± 2.9 (6)†††††
	MPCA	10.1 ± 0.4 (3)	12.1 ± 0.9 (6)	1.5 ± 0.2 (3)†	0.5 ± 0.2 (6)††

For experimental details see the text. Statistically significant effects of administration of glucose are indicated by *** $P < 0.001$; those of DCA or MPCA by † $P < 0.05$, †† $P < 0.01$; and those of hyperthyroidism by ††† $P < 0.001$. Tissues were sampled at 2 h after treatment. Total PDH activities (expressed as munits/unit of citrate synthase) were: kidney, control 202.3 ± 8.5; hyperthyroid, 168.1 ± 11.6; and liver, control 293.7 ± 18.3; hyperthyroid, 339.4 ± 21.7.

Table 2. Effects of MPCA and insulin on renal and hepatic PDH_a in DCA-treated, control and T₃-treated 48 h-starved rats

For experimental details see the text. Statistically significant effects of MPCA or insulin are indicated by: * $P < 0.05$, *** $P < 0.001$; and those of T₃ treatment by: † $P < 0.05$; †† $P < 0.01$; ††† $P < 0.001$.

Tissue	Treatment	PDH _a			
		Citrate synthase (units/g wet wt.)		(munits/unit of citrate synthase)	
		Control	T ₃ -treated	Control	T ₃ -treated
Kidney	Control	20.7 ± 0.7(9)	21.9 ± 0.5(8)	73.0 ± 5.1(9)	72.2 ± 8.7(8)
	MPCA	20.5 ± 0.8(8)	22.0 ± 1.5(5)	76.8 ± 4.0(8)	83.3 ± 8.1(5)
	Insulin	22.8 ± 0.9(8)	20.1 ± 0.6(4)†	72.4 ± 3.4(8)	98.8 ± 7.5(4)*††
Liver	Control	9.2 ± 0.7(9)	11.9 ± 0.6(8)†	25.1 ± 3.6(9)	97.6 ± 5.8(8)†††
	MPCA	9.4 ± 0.9(8)	12.7 ± 0.8(5)†	14.6 ± 2.8(8)*	77.4 ± 10.3(5)†††
	Insulin	10.8 ± 0.4(5)	12.9 ± 0.7(4)†	91.0 ± 8.3(5)***	85.2 ± 6.5(4)
				(% of total complex)	
				Control	T ₃ -treated
				36.1 ± 2.5(9)	42.9 ± 5.2(8)
				38.0 ± 2.0(8)	49.6 ± 4.8(5)†
				35.8 ± 1.6(8)	58.8 ± 4.4(4)†††
				8.5 ± 1.2(9)	28.8 ± 1.7(8)†††
				4.9 ± 1.0(8)*	22.8 ± 3.0(5)†††
				31.0 ± 2.8(5)***	25.1 ± 1.9(4)

phosphorylation of regulatory sites in the PDH complex (in heart, PDH re-activation is retarded by multi-site phosphorylation [5,6]) or a consequence of increased activity of PDH phosphatase.

Effects of insulin in dichloroacetate-treated rats

Insulin administration of DCA-treated euthyroid rats increased hepatic PDH_a activities to values observed in glucose-administered DCA-treated rats (Table 2). Unless insulin affects the uptake of DCA into liver mitochondria, a direct effect of the hormone to increase the rate of dephosphorylation of hepatic PDH is indicated. From the present results it is unclear whether this is achieved through specific activation of PDH phosphatase by an 'insulin mediator' ([28,29]; see under 'General discussion') or changes in the intramitochondrial concentration of one or more effectors of the phosphatase, as has been suggested to be the case for adipose-tissue PDH [25,26]. In DCA-treated hyperthyroid rats, insulin administration was without further effect on hepatic PDH_a activities. This finding argues against an effect of insulin to increase DCA transport into liver mitochondria. As liver PDH_a activities were similar in hyperthyroid and euthyroid rats treated with DCA, it is strongly suggested that hyperthyroidism mimics the effect of insulin to increase PDH phosphatase activity, although the mechanism of action of the two hormones is not of necessity identical.

Insulin administration had no effect on renal PDH activity in rats (euthyroid or hyperthyroid) treated with DCA. This supports the theory that, in kidney, the activity of PDH kinase is the major factor regulating the proportion of the complex in the active form and that insulin exerts no control on kinase activity in the kidney.

General discussion

In tissues which are not important sites of fatty acid synthesis (such as heart or kidney), acetyl-CoA produced from either lipid or carbohydrate fuels is utilized exclusively for energy production. The liver is unique in that it is a site of both fat synthesis and fat oxidation. In the fed state the reciprocal regulation of these two processes is achieved, at least in part, through changes in the rate of lipogenesis (reviewed in [30]). Citrate is the major lipogenic precursor, and, since citrate accumulation depends on the extent to which PDH flux exceeds flux through the citric acid cycle, acetyl-CoA produced from carbohydrate is committed to lipogenesis only after hepatic energy demands have been satisfied. A decrease in the rate of lipogenesis is associated with increased energy production from fat through decreased inhibition of mitochondrial fatty acid uptake by malonyl-CoA.

Insulin increases PDH activity [31] and flux through PDH [32] in hepatocytes from fed rats. The mechanism is unlikely to involve decreased activity of PDH kinase via changes in metabolite concentration ratios [31], but PDH-phosphate dephosphorylation may be increased through the production of a low- M_r mediator, possibly a peptide, which stimulates phosphatase activity [28,29]. Although effects of insulin or glucose to increase hepatic PDH *in vivo* in fed rats have previously been difficult to demonstrate [33], the present experiments provide evidence that re-feeding of carbohydrate after prolonged starvation leads to stimulation of hepatic PDH phosphatase, and PDH re-activation can be observed if kinase activity is decreased through inhibition by DCA. It seems

likely that adaptive increases in PDH kinase activities occurring in the liver in response to starvation (mimicked *in vitro* by provision of glucagon and fatty acid [4]) set the kinase/phosphatase activity ratio such that the effect of increased phosphatase is not apparent until substantial decreases in kinase concentrations have occurred. The situation in euthyroid liver is distinct from that in adipose tissues, where administration of glucose leads to rapid re-activation of the PDH complex [14,33,34] and increased lipogenesis from carbohydrate as precursor is concomitantly observed [14,33]. In this tissue, cogent evidence has been presented by Denton and co-workers that glucose re-feeding (through increased insulin concentrations) exerts its effects on PDH through increased PDH phosphatase activity [24,25,33,34]. In liver of hyperthyroid rats, increased rates of lipogenesis [9,17] are accompanied with more ready re-activation of PDH (Table 1), possibly through increased PDH phosphatase activity. Pyruvate carboxylase activity is also increased [20]. The increases in hepatic pyruvate metabolism elicited by hyperthyroidism, together with increased lactate and pyruvate provision from peripheral tissues [35], may make a significant contribution towards the increased glucose turnover and utilization observed in clinical and experimental hyperthyroidism.

Intragastric glucose re-feeding causes immediate re-activation of renal PDH in 48 h-starved euthyroid rats (Fig. 1*b*; [8]), but neither liver (Fig. 1*b*); [8]) nor cardiac [8] PDH activity is increased. Characteristics of the regulation of complex activity in these latter tissues, in addition to end-product inhibition and regulation of kinase by metabolite ratios, include long-term changes in concentration of kinase/kinase activator [3,4] and activation of phosphatase by $1\ \mu\text{M}\text{-Ca}^{2+}$ (reviewed in [26]). Further experiments are needed to find whether modulation of these aspects of PDH regulation in kidney may account for the difference in response of renal and cardiac or hepatic PDH to re-feeding: the present work, however, indicates that, in kidney, re-activation predominantly occurs through effects on the kinase, and highlights the importance of control of the relative activities of PDH kinase and PDH phosphatase in determining PDH phosphorylation state. A novel finding is that hyperthyroidism increases renal PDH activity in starvation via inhibition of PDH kinase. The question of whether this is the result of altered fuel metabolism within the tissue is an area for future research.

In summary, our results provide evidence for distinct modes of regulation of PDH in different tissues after carbohydrate re-feeding. Effects on PDH phosphatase may be related to a lipogenic function of the complex, whereas effects on PDH kinase may be related to its role in energy supply. The mechanisms of control of PDH in the liver are of particular interest, as in this tissue PDH has both an anabolic and an energetic role. The work also highlights the potential importance of tissue-specific modulation of the regulatory properties of PDH kinase and PDH phosphatase by thyroid status.

We thank the National Kidney Research Fund and the U.K. Medical Research Council for financial support, and Miss Angela Whibley for secretarial assistance.

REFERENCES

1. Randle, P. J., Fuller, S. J., Kerbey, A. L., Sale, G. T. & Vary, T. C. (1984) *Horm. Cell Regul.* **8**, 139–151
2. Wieland, O. H. (1983) *Rev. Physiol. Biochem. Pharmacol.* **96**, 123–170
3. Kerbey, A. L., Richardson, L. J. & Randle, P. J. (1984) *FEBS Lett.* **176**, 115–119
4. Fatania, H. R., Vary, T. C. & Randle, P. J. (1986) *Biochem. J.* **234**, 233–236
5. Sugden, P. H., Hutson, N. J., Kerbey, A. L. & Randle, P. J. (1978) *Biochem. J.* **169**, 433–435
6. Sale, G. J. & Randle, P. J. (1982) *Biochem. J.* **206**, 221–229
7. Caterson, I. D., Fuller, S. J. & Randle, P. J. (1982) *Biochem. J.* **208**, 53–60
8. Holness, M. J., French, T. J. & Sugden, M. C. (1986) *Biochem. J.* **235**, 441–445
9. Sugden, M. C., Steare, S. E., Watts, D. I. & Palmer, T. N. (1983) *Biochem. J.* **210**, 937–944
10. Sugden, M. C., Watts, D. I., Palmer, T. N. & Myles, D. D. (1983) *Biochem. Int.* **7**, 329–337
11. Newgard, C. B., Hirsch, L. J., Foster, D. W. & McGarry, J. D. (1983) *J. Biol. Chem.* **258**, 8046–8052
12. Newgard, C. B., Moore, S. V., Foster, D. W. & McGarry, J. D. (1984) *J. Biol. Chem.* **259**, 6958–6963
13. Katz, J. & McGarry, J. D. (1984) *J. Clin. Invest.* **74**, 1901–1909
14. Sugden, M. C., Watts, D. I., Marshall, C. E. & McCormack, J. G. (1982) *Biosci. Rep.* **2**, 289–297
15. Agius, L. & Williamson, D. H. (1980) *Biochem. J.* **190**, 477–480
16. Holness, M. J., Palmer, T. N. & Sugden, M. C. (1985) *Biochem. J.* **232**, 255–259
17. Sugden, M. C., Watts, D. I. & Marshall, C. E. (1981) *Biosci. Rep.* **1**, 757–764
18. Agius, L. & Williamson, D. H. (1981) *Biochim. Biophys. Acta* **666**, 127–132
19. Kerbey, A. L., Randle, P. J., Cooper, R. H., Whitehouse, S., Pask, H. T. & Denton, R. M. (1976) *Biochem. J.* **154**, 327–348
20. Weinberg, M. B. & Utter, M. F. (1979) *J. Biol. Chem.* **254**, 9492–9499
21. Axelrod, L., Lloyd-Jones, P. & Martin, D. B. (1979) *Diabetes* **28**, 651–657
22. Whitehouse, S., Cooper, R. H. & Randle, P. J. (1973) *Biochem. J.* **141**, 761–774
23. Vary, T. C. & Randle, P. J. (1984) *J. Mol. Cell. Cardiol.* **16**, 723–733
24. Denton, R. M., McCormack, J. G. & Marshall, S. E. (1984) *Biochem. J.* **217**, 441–452
25. Marshall, S. E., McCormack, J. G. & Denton, R. M. (1984) *Biochem. J.* **218**, 249–260
26. Denton, R. M. & McCormack, J. G. (1985) *Am. J. Physiol.* **249**, E543–E554
27. Dennis, S. C., De Buysere, M., Scholz, R. & Olson, M. S. (1978) *J. Biol. Chem.* **253**, 2229–2237
28. Parker, J. C. & Jarett, L. (1985) *Diabetes* **34**, 92–97
29. Macaulay, S. L. & Jarett, L. (1985) *Arch. Biochem. Biophys.* **237**, 142–150
30. Sugden, M. C. & Williamson, D. H. (1982) in *Metabolic Compartmentation* (Sies, H., ed.), pp. 287–315, Academic Press, New York and London
31. Assimacopoulos-Jeannet, F., McCormack, J. G., Prentki, M., Jeanrenaud, B. & Denton, R. M. (1982) *Biochim. Biophys. Acta* **717**, 86–90
32. Agius, L. & Vaartjes, W. J. (1985) *Biochim. Biophys. Acta* **844**, 393–399
33. Stansbie, D., Brownsey, R. W., Crettaz, M. & Denton, R. M. (1976) *Biochem. J.* **160**, 413–416
34. McCormack, J. D. & Denton, R. M. (1977) *Biochem. J.* **166**, 627–630
35. Okajima, F. & Ui, M. (1979) *Biochem. J.* **182**, 565–575