The contribution of pyruvate cycling to loss of [6-³H]glucose during conversion of glucose to glycogen in hepatocytes: effects of insulin, glucose and acinar origin of hepatocytes

Loranne AGIUS,* David TOSH† and Matthew PEAK

Department of Medicine, The Medical School, Framlington Place, The University of Newcastle upon Tyne, Newcastle upon Tyne NE2 4HH, U.K.

1. During conversion of [6-³H,U-¹⁴C]glucose to glycogen in liver, loss of 6-³H can occur either by cycling via pyruvate (between glycolysis and gluconeogenesis) or by other mechanisms. We used mercaptopicolinate, an inhibitor of phosphoenolpyruvate carboxykinase, to determine the extent to which pyruvate cycling contributes to loss of 6-³H during glucose conversion to glycogen in hepatocytes. 2. Mercaptopicolinate increased the ³H/¹⁴C ratio in glycogen during incubation of rat, guinea pig, pig and human hepatocytes with [6-³H,U-¹⁴C]glucose. The increase in the ³H/¹⁴C ratio in glycogen caused by mercaptopicolinate was greater in periportal than in perivenous rat hepatocytes, indicating that cycling of glucose via pyruvate is more prominent in cells with a higher gluconeogenic relative to glycolytic capacity. 3. The effect of mercaptopicolinate on the ³H/¹⁴C ratio in glycogen was observed both in the absence and in the presence of insulin, indicating that stimulation of glyco-

gen synthesis by insulin is not associated with inhibition of pyruvate cycling. In rat and guinea pig but not in pig hepatocytes, the effects of mercaptopicolinate on the ³H/¹⁴C ratio in glycogen were greater at 10–15 mM glucose than at 30 mM glucose, suggesting diminished cycling via pyruvate at high glucose concentrations. 4. Insulin increased the loss of 6-³H during stimulation of conversion of glucose to glycogen in hepatocytes from all species. This was due in part to an increase in pyruvate cycling and in part to other mechanisms that are not inhibited by mercaptopicolinate. 5. These results suggest that pyruvate cycling is a significant, but not exclusive, component of the loss of 6-³H in the hepatocyte during glucose conversion to glycogen. The extent of pyruvate cycling is dependent on the acinar origin of the hepatocytes and on the glucose concentration and presence of insulin.

INTRODUCTION

Hepatic glycogen can be synthesized either by a direct pathway from glucose involving conversion of glucose to glucose 6-phosphate to UDP-glucose and then to glycogen, or by an indirect pathway involving conversion of glucose to gluconeogenic precursors which are then converted to glucose 6-phosphate in the hepatocyte [1–4]. A variety of techniques have been used to estimate the relative contributions of these pathways *in vivo*. One technique involves the determination of the ³H/¹⁴C ratio in glycogen after infusion of [6-³H,U-¹⁴C]glucose [5–8]. This is based on the assumption that ³H label in the 6-position of glucose is lost when pyruvate formed from [6-³H]glucose is reconverted to glucose 6-phosphate [1,6,9,10]. Consequently, the further the ratio of ³H/¹⁴C in glycogen relative to that in the plasma glucose is from unity, the greater is the extent of cycling of glucose via pyruvate and thereby the contribution of the indirect pathway [1]. It is widely assumed that the contribution of the indirect pathway as determined *in vivo* represents conversion of glucose to gluconeogenic precursors in extrahepatic tissues and the reconversion of these gluconeogenic precursors to glucose 6-phosphate and then to glycogen within the liver. However, when rat or mouse hepatocytes are incubated with [6-³H,¹⁴C]glucose, the ratio of ³H/¹⁴C incorporated into glycogen relative to that in glucose is significantly less than unity (0.5–0.7), indicating that substantial loss of 6-³H occurs within the hepatocyte during conversion of glucose to glycogen [11,12]. One explanation for this loss of 6-³H during glucose conversion to

glycogen is that it represents substrate cycling between glycolysis (conversion of glucose 6-phosphate to pyruvate) and reconversion of pyruvate to glucose 6-phosphate [11]. A recent comment by Landau [13], however, points out that theoretically there may be other mechanisms by which ³H in the 6-position of glucose can be lost. These are at the level of UDP-glucose, by reversible dehydrogenation to UDP-glucuronate, by a transaldolase reaction between fructose 6-phosphate and glyceraldehyde 3-phosphate, and at the level of triose phosphate [13]. If loss of 6-³H by label-exchange reactions at these sites occurs to a significant extent relative to cycling via pyruvate, then the ratio of ³H/¹⁴C incorporated into glycogen would overestimate the contribution of pyruvate cycling. The extent by which loss of 6-³H during glucose conversion to glycogen in hepatocytes occurs as a result of pyruvate cycling as distinct from other reactions not involving pyruvate cycling has not been determined. It remains debatable whether these additional mechanisms account for a negligible [14] or significant [13] proportion of the total loss of 6-³H.

In the present study we determined the incorporation of [6-³H,U-¹⁴C]glucose into glycogen in hepatocytes isolated from different acinar zones and from different species and we used mercaptopicolinate, an inhibitor of phosphoenolpyruvate carboxykinase [15–17] and thereby of conversion of pyruvate to phosphoenolpyruvate, to estimate to what extent loss of 6-³H occurs by cycling via pyruvate. We demonstrate that pyruvate cycling is a significant but not exclusive component of loss of 6-³H during glucose conversion to glycogen in hepatocytes and that

Abbreviations used: PP, periportal; PV, perivenous.

* To whom correspondence should be addressed.

† Present address: Department of Biochemical Medicine, University of Dundee, Dundee DD1 4HN, U.K.

it is dependent on the glucose concentration and the presence of insulin. Furthermore, we demonstrate that the extent of glucose cycling via pyruvate during conversion of glucose to glycogen is greater in periportal hepatocytes, which have a higher gluconeogenic capacity, than in perivenous hepatocytes; this suggests that the gluconeogenic rather than the glycolytic capacity is the main determinant of the extent of glucose cycling via pyruvate within the hepatocyte.

MATERIALS AND METHODS

Materials

[U-¹⁴C]Glucose and [3-³H]glucose were from Amersham International (Amersham, Bucks., U.K.). [6-³H]Glucose and [3-¹⁴C]pyruvate were from New England Nuclear (Boston, MA, U.S.A.). Insulin and collagenase (type IV) were from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Mercaptopicolinic acid was from SmithKline and French (Philadelphia, PA, U.S.A.). Minimum essential medium with Earle's salts and 20 mM Hepes was from Northumbria Biologicals (Cramlington, Northumberland, U.K.). Sources of other materials were as described previously [18,19].

Hepatocyte sources

Rats were of the Wistar strain (male; body wt. 240–300 g) and were bred in the Animal House, University of Newcastle upon Tyne. Guinea pigs were of the Dunkin–Hartley strain (male; body wt. 260–340 g) and were obtained from Bantin and Kingman, Hull, U.K. Pigs (male; Large White) were 3–5 weeks old and were obtained from Cockle Park, University of Newcastle upon Tyne. Human liver was from brain-dead kidney donors (male, 65 year and 69 year).

Hepatocyte isolation

Rat periportal and perivenous hepatocytes were isolated by the digitonin–collagenase perfusion technique as in [20]. Guinea pig hepatocytes were isolated by collagenase perfusion of whole liver [21]. Pig and human hepatocytes were isolated by collagenase perfusion of a 150–200 g wedge biopsy [22].

Enzyme activities

Alanine aminotransferase, fructose biphosphatase and pyruvate kinase activities in rat periportal (PP) and perivenous (PV) hepatocytes were determined as described previously [20,22] and were (munits/mg of protein; means $\pm$ SEM; $n = 5$): alanine aminotransferase, PP, 215 ± 31 ; PV, 130 ± 9 ; fructose biphosphatase, PP, 46 ± 4 , PV, 35 ± 2 ; pyruvate kinase, PP, 332 ± 52 ; PV, 354 ± 15 ; alanine aminotransferase/pyruvate kinase, PP, 0.62 ± 0.16 ; PV, 0.37 ± 0.02 ; pyruvate kinase/fructose biphosphatase, PP, 7.5 ± 0.8 ; PV, 10.1 ± 0.3 .

Hepatocyte monolayers

The hepatocytes were suspended in minimum essential medium containing 5% (v/v) fetal bovine serum and inoculated in Corning 24-well plates at a density of 6.4×10^4 cells/cm². After cell attachment (approx. 6 h) the medium was changed to serum-free minimum essential medium containing 10 nM dexamethasone phosphate and 5 mM glucose, and the hepatocyte monolayers were cultured for approx. 16 h before determination of gluconeogenesis [20] or glycogen synthesis [19].

Incubation conditions and analytical methods

For determination of gluconeogenesis and glycogen synthesis, hepatocyte monolayers were incubated in fresh minimum essential medium without added glutamine and with the substrate supplements and other additions indicated. The medium contained either 5 mM glucose or was supplemented with additional glucose up to the concentrations indicated. Incubations with [3-¹⁴C]pyruvate (1 mM) were as described previously [20]. Incubations for determination of glucose cycling contained [U-¹⁴C]glucose (1.5 μ Ci/ml) and either [6-³H]glucose or [3-³H]glucose (4.5 μ Ci/ml). Mercaptopicolinate was dissolved in 150 mM Hepes at $100 \times$ final concentration and control incubations received an equivalent volume of buffer. Incubations were performed in either the absence or the presence of 10 nM insulin. This concentration of insulin causes maximum stimulation of glycogen deposition during 3 h incubations [19]. Half-maximum stimulation occurs at a concentration of ~ 0.2 nM and maximum stimulation at 1 nM–10 nM insulin [19].

Incubations with [3-¹⁴C]pyruvate were for 2 h, and all other incubations for determination of glycogen synthesis were for 3 h. On termination of the incubations the hepatocyte monolayers were washed once with 150 mM NaCl and extracted in 0.1 M NaOH [19]. Cell glycogen was determined enzymically after digestion with amyloglucosidase and fluorometric determination of the glucose formed [19]. The glucose and lactate concentrations in the medium were determined enzymically after deproteinization of the medium with perchloric acid [19]. The incorporation of [¹⁴C]pyruvate into glucose was determined as in [20]. The incorporation of radiolabel into glycogen was determined after ethanol precipitation of the glycogen as described previously [19]. The ³H and ¹⁴C d.p.m. in glycogen and glucose were determined simultaneously using a Beckman LS1801 liquid scintillation counter using appropriate channels.

Cell volume was determined from the accumulation of 3-*O*-methyl[¹⁴C]glucose. Hepatocytes were incubated for 60 min in glucose-free balanced salts medium containing 10 mM 3-*O*-methyl[¹⁴C]glucose, as in [18]. Cell volume determined by this technique represents the glucose-permeant space and is expressed as μ l per mg of cell protein [18].

Cell protein was determined by an automated Lowry method [23].

Expression of results

Gluconeogenesis in experiments with [¹⁴C]pyruvate is expressed as nmol of [¹⁴C]pyruvate incorporated into glucose/h per mg of cell protein, determined from the specific radioactivity of pyruvate in the medium. Gluconeogenesis in incubations with dihydroxyacetone or glycerol represents the increase in glucose concentration in the medium during the 3 h incubation relative to that in control incubations without these substrates, expressed as nmol of glucose formed/3 h per mg of protein. Glycogen synthesis represents the incorporation of [U-¹⁴C]glucose into glycogen and is expressed as nmol of [¹⁴C]glucose incorporated in glycogen/3 h per mg of protein, and was determined from the specific radioactivity of glucose in the medium which was constant during the incubation. Glycogen deposition represents the increment in cell glycogen during a 3 h incubation with the glucose concentration indicated, determined by subtracting the glycogen content at the start of the incubation from the glycogen content after 3 h, and is expressed as nmol of glucosyl units deposited as glycogen/mg of cell protein. The ³H/¹⁴C ratio in glycogen after incubation with either [6-³H,U-¹⁴C]glucose or [3-³H,U-¹⁴C]glucose is expressed relative to the ratio of ³H/¹⁴C in glucose in the medium i.e. [³H/¹⁴C]glycogen/[³H/¹⁴C]glucose. Time

Figure 1 Effects of mercaptopicolinate on gluconeogenesis and glycogen synthesis

(a) Effects of mercaptopicolinate on incorporation of 1 mM [$3\text{-}^{14}\text{C}$]pyruvate into glucose in rat hepatocytes. Values are expressed as nmol of [^{14}C]pyruvate incorporated/h per mg of protein, and are means $\pm$ S.E.M. of four experiments. (b) and (c) Effects of mercaptopicolinate on incorporation of 15 mM [$\text{U-}^{14}\text{C}$]glucose into glycogen in the absence of insulin (○) or in the presence of 10 nM insulin (●). Values are means $\pm$ S.E.M. of three experiments for rat hepatocytes (b) and means of two experiments for guinea pig hepatocytes (c), and are expressed as nmol of [^{14}C]glucose incorporated/3 h per mg of protein.

courses of glycogen deposition and [^{14}C]glucose incorporation into glycogen were linear during 3 h. The $^3\text{H}/^{14}\text{C}$ ratio in glycogen determined after 2, 3 and 4 h of incubation was constant.

Estimates of the direct pathway and indirect pathway of glycogen synthesis from glucose, not taking into account loss of $6\text{-}^3\text{H}$ by label-exchange reactions, were determined from the incorporation of [$6\text{-}^3\text{H}$]glucose into glycogen (direct pathway) and from the difference between ^{14}C and $6\text{-}^3\text{H}$ incorporation into glycogen (indirect pathway). The proportion by which pyruvate cycling contributes to the indirect pathway was estimated from the increment in $^3\text{H}/^{14}\text{C}$ ratio caused by mercaptopicolinate (MP) as a proportion of the deviation of the control ratio from unity, i.e. $(^3\text{H}/^{14}\text{C}_{\text{MP}} - ^3\text{H}/^{14}\text{C}_{\text{control}}) / (1 - ^3\text{H}/^{14}\text{C}_{\text{control}}) \times 100$. The underlying assumptions are discussed in the Results section.

Values are means $\pm$ SEM for the numbers of cell preparations (experiments) indicated. Statistical analysis for differences in the presence of mercaptopicolinate or insulin was by the paired t test.

RESULTS

Effects of mercaptopicolinate on gluconeogenesis and glycogen synthesis

Mercaptopicolinate inhibits the conversion of pyruvate to glucose [15], and this is presumed to be due to inhibition of phosphoenolpyruvate carboxykinase [16,17]. It also stimulates glycogen synthesis from glucose in fetal rat hepatocytes [24] and from substrates that enter the gluconeogenic pathway at the level of triose phosphates in adult rat hepatocyte suspensions [25,26]. We determined the effects of mercaptopicolinate (0.25–2 mM) on the incorporation of [$3\text{-}^{14}\text{C}$]pyruvate into glucose (Figure 1a) and glycogen in rat hepatocytes, and on the incorporation of [$\text{U-}^{14}\text{C}$]glucose into glycogen in rat and guinea pig hepatocytes (Figures 1b and 1c). Mercaptopicolinate inhibited the incorporation of [$3\text{-}^{14}\text{C}$]pyruvate into glucose by 57% at 0.25 mM and by 79–88% at 0.5–2 mM (Figure 1a), and it inhibited the incorporation of [^{14}C]pyruvate into glycogen by a similar percentage (55%, 77%, 80% and 86% inhibition at 0.25, 0.5, 1.0 and 2.0 mM mercaptopicolinate respectively). In contrast with the inhibition of incorporation of [^{14}C]pyruvate into glycogen, mercaptopicolinate stimulated the incorporation of [^{14}C]glucose into glycogen (Figures 1b and 1c). The effect was biphasic: in the

rat significant stimulation occurred at concentrations of 0.25–1 mM (Figure 1b), whereas in the guinea pig significant stimulation occurred at 0.25 mM mercaptopicolinate (Figure 1c).

Correlation between glycogen deposition and [^{14}C]glucose incorporation into glycogen

When hepatocytes were pre-cultured in minimum essential medium containing 5 mM glucose and then incubated for 3 h in fresh medium containing 10–30 mM [$\text{U-}^{14}\text{C}$]glucose, the net increment in cell glycogen during the 3 h incubation correlated with the incorporation of [$\text{U-}^{14}\text{C}$]glucose into glycogen (Figure 2a). Glycogen deposition increased with increasing glucose concentration, and was increased further by insulin (10 nM) at all glucose concentrations (Figure 2a). This correlation between glycogen deposition and incorporation of [$\text{U-}^{14}\text{C}$]glucose into glycogen suggests that the latter represents net glycogen synthesis from glucose, and therefore the sum of glycogen synthesis by the direct and indirect pathways. Glycogen deposition and [^{14}C]glucose incorporation into glycogen also correlated in incubations with mercaptopicolinate (Figure 2b), indicating that the stimulation of glycogen deposition by mercaptopicolinate can be accounted for by stimulation of glucose conversion to glycogen.

Effects of mercaptopicolinate on the metabolism of dihydroxyacetone and glycerol

The mechanism by which mercaptopicolinate stimulates glycogen synthesis from glucose ([24] and Figures 1b, 1c and 2b) and from substrates that enter the gluconeogenic pathway at the level of triose phosphate [25,26] has not been established. Two hypotheses have been proposed. One is that mercaptopicolinate inhibits glucose-6-phosphatase and thereby causes diversion of glucose 6-phosphate to glycogen [27,28]. The other is that mercaptopicolinate causes cell swelling and thereby mimics the effects of amino acids and hypo-osmotic swelling, which increase glycogen synthesis via an increase in cell volume [26].

The validity of the use of mercaptopicolinate to determine the extent by which loss of $6\text{-}^3\text{H}$ from glucose represents cycling via pyruvate rests on the assumption that mercaptopicolinate inhibits the conversion of pyruvate to glucose 6-phosphate. If

Figure 2 Correlation between [^{14}C]glucose incorporation into glycogen and glycogen deposition

(a) Hepatocytes were incubated with the glucose concentrations (10–30 mM) indicated in parentheses in either the absence (○) or presence (□) of insulin. Results are means $\pm$ S.E.M. of five experiments. (b) Hepatocytes were incubated with 10 mM glucose in either the absence (○) or presence of insulin (□), mercaptopicolinate (●) or insulin + mercaptopicolinate (■). Results are means $\pm$ S.E.M. of nine experiments. Values are expressed as nmol of [^{14}C]glucose incorporated into glycogen/3 h per mg of protein versus nmol of glucosyl units deposited as glycogen/3 h per mg of protein.

mercaptopicolinate inhibits glucose 6-phosphatase [27] more potently than it inhibits phosphoenolpyruvate carboxykinase [16,17], it would influence the $^3\text{H}/^{14}\text{C}$ ratio in glycogen by altering the partitioning of [^{14}C]glucose 6-phosphate between glycogen synthesis and glucose formation rather than by inhibition of pyruvate conversion to glucose 6-phosphate. The finding that mercaptopicolinate causes a similar percentage inhibition of [^{14}C]pyruvate incorporation into glucose and glycogen, despite simultaneous stimulation of [^{14}C]glucose incorporation into glycogen (Figure 1b), indicates that mercaptopicolinate inhibits pyruvate metabolism before it affects formation of glucose 6-phosphate, and consequently that phosphoenolpyruvate carboxykinase is more sensitive to inhibition by mercaptopicolinate than is glucose 6-phosphatase. This is consistent with cross-over plots of glycolytic/gluconeogenic intermediates in liver perfusions with alanine and 0.1 mM mercaptopicolinate [29].

Although a role for glucose-6-phosphatase in the inhibition of pyruvate metabolism by mercaptopicolinate can be excluded, the possibility remains that stimulation of glycogen synthesis from glucose (Figure 1b) or dihydroxyacetone [27] by mercaptopicolinate could be due to inhibition of glucose-6-phosphatase. To investigate this possibility we determined the effects of mercaptopicolinate on glycogen deposition and glucose production in rat hepatocytes incubated with dihydroxyacetone or glycerol (Table 1). Mercaptopicolinate (1 mM) increased gly-

Table 1 Effects of mercaptopicolinate on glycogen deposition and glucose and lactate production in incubations with dihydroxyacetone or glycerol

Hepatocytes were incubated for 3 h in minimum essential medium containing 5 mM glucose and either 10 mM dihydroxyacetone or 10 mM glycerol, and without or with 1 mM mercaptopicolinate (MP) or 10 nM insulin as indicated. Glucose and lactate production, determined from the increase in concentration in the medium, are expressed as nmol formed/3 h per mg of protein. Glycogen deposition is expressed as nmol of glucosyl units deposited/3 h per mg of protein. Values are means $\pm$ S.E.M. for each of five cultures. Statistics: * $P < 0.05$, ** $P < 0.005$, with versus without insulin; † $P < 0.05$, †† $P < 0.005$, with versus without mercaptopicolinate.

Additions	Glucose production (nmol/3 h per mg)	Lactate production (nmol/3 h per mg)	Glycogen formation (nmol/3 h per mg)
10 mM Dihydroxyacetone	1337 $\pm$ 229	1134 $\pm$ 95	183 $\pm$ 7
+ insulin	1001 $\pm$ 42	1261 $\pm$ 111*	322 $\pm$ 32*
+ MP	1446 $\pm$ 244	1025 $\pm$ 88††	267 $\pm$ 17†
+ MP + insulin	1335 $\pm$ 277	1100 $\pm$ 112†	376 $\pm$ 47
10 mM Glycerol	1275 $\pm$ 207	506 $\pm$ 58	86 $\pm$ 5
+ insulin	1249 $\pm$ 130	811 $\pm$ 80*	216 $\pm$ 54**
+ MP	1500 $\pm$ 273	557 $\pm$ 66†	160 $\pm$ 10††
+ MP + insulin	1421 $\pm$ 212	862 $\pm$ 86*†	285 $\pm$ 44*

Table 2 Effects of mercaptopicolinate on lactate formation in incubations with glucose

Hepatocytes were incubated for 3 h in minimum essential medium containing the glucose concentrations indicated without or with 1 mM mercaptopicolinate (MP) or 10 nM insulin as indicated. Lactate production, determined from the increase in concentration in the medium, is expressed as nmol formed/3 h per mg of protein. Values are means $\pm$ S.E.M. for each of five cultures. Statistics: * $P < 0.05$, with versus without insulin; † $P < 0.05$, with versus without mercaptopicolinate.

Additions	Insulin	Lactate formation (nmol/3 h per mg of protein)	
		— MP	+ MP
[Glucose] (mM)			
5	—	113 $\pm$ 12	94 $\pm$ 14
5	+	208 $\pm$ 19*	131 $\pm$ 27†
15	—	319 $\pm$ 57	304 $\pm$ 66
15	+	466 $\pm$ 41*	397 $\pm$ 53†
30	—	518 $\pm$ 65	470 $\pm$ 78
30	+	686 $\pm$ 64*	570 $\pm$ 63†

cogen deposition from both dihydroxyacetone and glycerol as substrates, and it suppressed lactate production in incubations with dihydroxyacetone but increased lactate production from glycerol (Table 1). Mercaptopicolinate did not inhibit glucose formation with either substrate, suggesting that the increased glycogen deposition is probably not due to inhibition of glucose-6-phosphatase.

In incubations with glucose alone, mercaptopicolinate had no significant effect on lactate formation in the absence of insulin; however, it inhibited the stimulation of lactate formation caused by insulin (Table 2), indicating that it selectively increases glucose conversion to glycogen (Figure 1b) but not conversion of glucose to lactate.

Incubation of rat hepatocytes with mercaptopicolinate (0.25–1.0 mM) for 60 min was associated with an increase in cell volume (control, 2.00 ± 0.16 ; 0.25 mM, 2.17 ± 0.13 ; 0.50 mM, 2.20 ± 0.11 ; 1.0 mM, 2.08 ± 0.14 $\mu\text{l}/\text{mg}$ of protein; means $\pm$ S.E.M.; $n = 4$; $P < 0.05$, 1 mM versus control), in agreement

Table 3 Effects of insulin and mercaptopicolinate on the incorporation of [6-³H,U-¹⁴C]glucose and [3-³H,U-¹⁴C]glucose into glycogen in rat PP and PV hepatocytes

Hepatocytes were incubated for 3 h in minimum essential medium containing the glucose concentrations indicated and either [6-³H,U-¹⁴C]glucose or [3-³H,U-¹⁴C]glucose in the absence or presence of 10 nM insulin or 1 mM mercaptopicolinate (MP). Glycogen synthesis represents the incorporation of [¹⁴C]glucose into glycogen and is expressed as nmol of [¹⁴C]glucose incorporated/3 h per mg of protein. The ³H/¹⁴C label incorporated into glycogen is expressed as a proportion of the ³H/¹⁴C activity in glucose in the medium. Values are means $\pm$ S.E.M. for each of five cultures, except for the experiments with [3-³H,U-¹⁴C]glucose which are from each of three cultures. Statistics: * $P < 0.05$, ** $P < 0.005$, with versus without insulin; † $P < 0.05$, †† $P < 0.005$, with versus without mercaptopicolinate.

[Glucose] (nM)	Hepatocytes		Glycogen synthesis (nmol of [¹⁴ C]glucose/3 h per mg)		³ H/ ¹⁴ C in glycogen [6- ³ H,U- ¹⁴ C]Glucose		³ H/ ¹⁴ C in glycogen [3- ³ H,U- ¹⁴ C]Glucose	
	Insulin	MP	PP	PV	PP	PV	PP	PV
10	—	—	14 $\pm$ 1	13 $\pm$ 2	0.80 $\pm$ 0.02	0.69 $\pm$ 0.03	0.57 $\pm$ 0.02	0.46 $\pm$ 0.02
10	+	—	27 $\pm$ 3*	27 $\pm$ 2*	0.75 $\pm$ 0.02*	0.65 $\pm$ 0.02	0.54 $\pm$ 0.03	0.48 $\pm$ 0.01
10	—	+	29 $\pm$ 4††	36 $\pm$ 9†	0.95 $\pm$ 0.02††	0.73 $\pm$ 0.03	0.55 $\pm$ 0.03	0.50 $\pm$ 0.03
10	+	+	49 $\pm$ 7**†	50 $\pm$ 7†	0.85 $\pm$ 0.02*†	0.67 $\pm$ 0.05	0.52 $\pm$ 0.02	0.50 $\pm$ 0.01
15	—	—	46 $\pm$ 5	50 $\pm$ 9	0.80 $\pm$ 0.02	0.68 $\pm$ 0.02	0.51 $\pm$ 0.02	0.49 $\pm$ 0.02
15	+	—	76 $\pm$ 8**	89 $\pm$ 6*	0.73 $\pm$ 0.02*	0.66 $\pm$ 0.02	0.48 $\pm$ 0.02	0.50 $\pm$ 0.01
15	—	+	71 $\pm$ 8††	78 $\pm$ 16†	0.92 $\pm$ 0.01††	0.73 $\pm$ 0.02†	0.52 $\pm$ 0.02	0.48 $\pm$ 0.03
15	+	+	110 $\pm$ 9**††	133 $\pm$ 14*	0.78 $\pm$ 0.02**	0.70 $\pm$ 0.01†	0.50 $\pm$ 0.01	0.48 $\pm$ 0.01
30	—	—	160 $\pm$ 11	186 $\pm$ 23	0.80 $\pm$ 0.02	0.69 $\pm$ 0.01	0.51 $\pm$ 0.01	0.49 $\pm$ 0.01
30	+	—	215 $\pm$ 16*	257 $\pm$ 22*	0.76 $\pm$ 0.01	0.68 $\pm$ 0.01	0.48 $\pm$ 0.02	0.47 $\pm$ 0.01
30	—	+	207 $\pm$ 17	242 $\pm$ 34†	0.86 $\pm$ 0.01†	0.70 $\pm$ 0.02	0.49 $\pm$ 0.02	0.49 $\pm$ 0.01
30	+	+	225 $\pm$ 23	341 $\pm$ 28**††	0.76 $\pm$ 0.01**	0.68 $\pm$ 0.02	0.49 $\pm$ 0.02	0.50 $\pm$ 0.01

with previous findings [26]. Since cell swelling increases glycogen synthesis from glucose and also from dihydroxyacetone as substrates [18,26], the increased glycogen synthesis from these substrates caused by mercaptopicolinate (Figure 1b and Table 1) could be related to cell swelling.

We infer from these results that the inhibition by mercaptopicolinate (0.25–1.0 mM) of pyruvate incorporation into glucose and glycogen is due to inhibition of pyruvate metabolism before the formation of glucose 6-phosphate, and presumably therefore at the level of phosphoenolpyruvate carboxykinase. Furthermore, the stimulation of glycogen synthesis from glucose, dihydroxyacetone and glycerol is also not a result of inhibition of glucose-6-phosphatase, but may be related to cell swelling.

Incorporation of [6-³H,U-¹⁴C]glucose into glycogen in rat PP and PV hepatocytes

Since mercaptopicolinate inhibits the conversion of pyruvate to glucose 6-phosphate, and presumably therefore glucose cycling via pyruvate, we determined the effects of mercaptopicolinate on the ratio of ³H/¹⁴C in glycogen relative to that in medium glucose during incubation with [6-³H,U-¹⁴C]glucose (Table 3). Mercaptopicolinate increased the incorporation of [¹⁴C]glucose into glycogen at all glucose concentrations. The stimulation in the combined presence of insulin and mercaptopicolinate was additive at 10 mM and 15 mM glucose, but not at 30 mM glucose (Table 3).

In the absence of mercaptopicolinate, the ratio of ³H/¹⁴C in glycogen relative to that in [6-³H,U-¹⁴C]glucose was not affected by increasing the glucose concentration from 10 mM to 30 mM for either PP or PV hepatocytes. The ratio of ³H/¹⁴C in glycogen was decreased by insulin and increased by mercaptopicolinate. The effect of mercaptopicolinate was greater in PP than in PV hepatocytes, was higher at low than at high glucose concentrations, and at 10 mM glucose was significant in both the absence and the presence of insulin (Table 3).

Incorporation of [3-³H,U-¹⁴C]glucose into glycogen

During conversion of [3-³H,U-¹⁴C]glucose into glycogen, 3-³H is lost at the level of triose phosphate and also via the pentose phosphate pathway [1]. To establish whether mercaptopicolinate affects the loss of ³H at the level of triose phosphate, we determined the effects of mercaptopicolinate on incorporation of [3-³H,U-¹⁴C]glucose into glycogen. Loss of 3-³H was greater than loss of 6-³H (Table 3), in agreement with some previous studies [6,30], although differences are not always observed [30]. Mercaptopicolinate did not affect the ³H/¹⁴C ratio in glycogen formed from [3-³H,U-¹⁴C]glucose under any of the experimental conditions (Table 3).

Incorporation of [6-³H,U-¹⁴C]glucose into glycogen in guinea pig, pig and human hepatocytes

In guinea pig and pig hepatocytes, glycogen synthesis was stimulated by insulin but not by 1 mM mercaptopicolinate (Table 4). In guinea pig hepatocytes the ratio of ³H/¹⁴C in glycogen relative to that in medium [6-³H,U-¹⁴C]glucose was decreased by insulin and increased by mercaptopicolinate. The latter effect was greater at low than at high glucose concentrations. In two experiments using guinea pig hepatocytes incubated with [3-³H,U-¹⁴C]glucose, mercaptopicolinate did not affect the ³H/¹⁴C ratio in glycogen relative to that in medium glucose (results not shown), similar to the findings in rat hepatocytes (Table 3). In pig hepatocytes the ³H/¹⁴C ratio in glycogen was also decreased by insulin and increased by mercaptopicolinate, but the ratio increased with increasing glucose concentration ($P < 0.01$, 30 mM versus 10 mM glucose). In human hepatocytes the ratio of ³H/¹⁴C in glycogen relative to that in [6-³H,U-¹⁴C]glucose at 10 mM glucose was also decreased by insulin (control, 0.79; +insulin, 0.65; means; $n = 2$) and increased by mercaptopicolinate (1 mM mercaptopicolinate, 0.94; +insulin, 0.77), indicating similar trends as for rat, guinea pig and pig hepatocytes.

Table 4 Effects of insulin and mercaptopicolinate on the incorporation of [6-³H,U-¹⁴C]glucose into glycogen in guinea pig and pig hepatocytes

Experimental details were as in Table 3. Values are means $\pm$ S.E.M. for four and three cultures for guinea pig and pig hepatocytes respectively. Statistics: * $P < 0.05$, with versus without insulin; † $P < 0.05$, †† $P < 0.005$, with versus without mercaptopicolinate (MP).

[Glucose] (mM)	Hepatocytes		Glycogen synthesis (nmol of [¹⁴ C]glucose/3 h per mg)		³ H/ ¹⁴ C in glycogen [6- ³ H,U- ¹⁴ C]Glucose	
	Insulin	MP	Guinea pig	Pig	Guinea pig	Pig
10	—	—	13 $\pm$ 3	12 $\pm$ 1	0.76 $\pm$ 0.04	0.79 $\pm$ 0.02
10	+	—	56 $\pm$ 12*	60 $\pm$ 18*	0.72 $\pm$ 0.07	0.73 $\pm$ 0.03*
10	—	+	9 $\pm$ 2	9 $\pm$ 1†	0.91 $\pm$ 0.04†	0.99 $\pm$ 0.07†
10	+	+	24 $\pm$ 6*†	38 $\pm$ 14†	0.83 $\pm$ 0.08†	0.92 $\pm$ 0.06†
15	—	—	25 $\pm$ 4	24 $\pm$ 4	0.76 $\pm$ 0.06	0.81 $\pm$ 0.02
15	+	—	110 $\pm$ 26*	84 $\pm$ 25*	0.71 $\pm$ 0.06*	0.72 $\pm$ 0.02*
15	—	+	16 $\pm$ 4	19 $\pm$ 5†	0.90 $\pm$ 0.06†	0.99 $\pm$ 0.03††
15	+	+	46 $\pm$ 10*	53 $\pm$ 21††	0.79 $\pm$ 0.01†	0.93 $\pm$ 0.06†
30	—	—	90 $\pm$ 18	48 $\pm$ 3	0.75 $\pm$ 0.08	0.89 $\pm$ 0.02
30	+	—	222 $\pm$ 41*	137 $\pm$ 30*	0.71 $\pm$ 0.07*	0.78 $\pm$ 0.04*
30	—	+	57 $\pm$ 12	37 $\pm$ 4	0.84 $\pm$ 0.09†	1.05 $\pm$ 0.07†
30	+	+	122 $\pm$ 13*	99 $\pm$ 26††	0.78 $\pm$ 0.08††	0.88 $\pm$ 0.01*†

The contribution of pyruvate cycling to loss of 6-³H

If it is assumed that incorporation of [¹⁴C]glucose into glycogen represents the sum of the direct and indirect pathways (since it correlates with glycogen deposition; Figure 2) and that incorporation of [6-³H]glucose into glycogen represents the direct pathway and the difference between ¹⁴C and ³H incorporation represents indirect pathways involving cycling via pyruvate as well as other substrate cycles, then it can be estimated that mercaptopicolinate stimulates the direct pathway in rat hepatocytes by as much as 2–2.5-fold at 10 mM glucose (9–11 increased to 26–28 nmol/3 h per mg) and 15 mM glucose (34–37 increased to 57–65 nmol/3 h per mg). However, mercaptopicolinate inhibits the direct pathway in guinea pig hepatocytes by 17–24% (9.9 decreased to 8.2 μ mol/3 h per mg at 10 mM glucose and 19 decreased to 14.4 nmol/3 h per mg at 15 mM glucose) but has little or no effect in pig hepatocytes (9.5 versus 8.9 nmol/3 h per mg at 10 mM glucose and 19.4 versus 18.8 nmol/3 h per mg at 15 mM glucose). These estimates of the direct pathway from the incorporation of [6-³H]glucose assume that 6-³H is lost only by substrate cycling, and might therefore be underestimates if 6-³H is lost by label-exchange reactions at the level of UDP-glucose [13]. The 2-fold increase in incorporation of [6-³H]glucose caused by mercaptopicolinate in rat hepatocytes is larger than can be accounted for by inhibition of label-exchange reactions, suggesting that mercaptopicolinate does stimulate the direct pathway. In view of these effects of mercaptopicolinate on the direct pathway in rat hepatocytes, it cannot be assumed that the change in the rate of the indirect pathway in the presence of mercaptopicolinate is exclusively due to inhibition of phosphoenolpyruvate carboxykinase. The effect of mercaptopicolinate on the indirect pathway in rat (and also guinea pig) hepatocytes can therefore only be estimated in relative terms, i.e. from the change in ³H/¹⁴C ratio relative to the deviation of the control ratio from unity (Table 5). These estimates assume that mercaptopicolinate does not alter the partitioning between direct and indirect pathways other than through inhibition of phosphoenolpyruvate carboxykinase. Since the stimulation of glycogen synthesis by mercaptopicolinate

Table 5 Pyruvate cycling relative to loss of 6-³H during conversion of [6-³H,U-¹⁴C]glucose into glycogen in rat, guinea pig and pig hepatocytes

Pyruvate cycling was determined from the increment in ³H/¹⁴C activity in glycogen caused by mercaptopicolinate (MP) and is expressed as a percentage of the difference of the control ratio from unity: [³H/¹⁴C_{MP} – ³H/¹⁴C_{control}]/(1 – ³H/¹⁴C_{control}) $\times$ 100. Values are calculated from the data in Tables 3 and 4.

[Glucose] (mM)	Insulin	Pyruvate cycling (% loss of 6- ³ H)			
		Rat PP	Rat PV	Guinea pig	Pig
10	—	75	13	63	95
10	+	40	6	39	70
15	—	60	16	58	95
15	+	19	12	28	75
30	—	30	3	36	100
30	+	0	0	24	46

could be due to cell swelling, we determined the effect of hypo-osmotic swelling (220 mosM versus 300 mosM) on the ³H/¹⁴C ratio in glycogen during incubation with [6-³H,U-¹⁴C]glucose, to establish whether changes in cell volume could account for the increase in the ratio. The ³H/¹⁴C ratio incorporated into glycogen was not affected by a hypo-osmolar medium (iso-osmolar, 0.81 $\pm$ 0.05; hypo-osmolar, 0.82 $\pm$ 0.05; means $\pm$ S.E.M., $n = 4$) which results in a 14% increase in cell volume [18]. This suggests that the increase in ratio caused by mercaptopicolinate is unlikely to be due to cell swelling.

The estimates in Table 5 for pyruvate cycling relative to loss of 6-³H show that, in the rat, pyruvate cycling relative to loss of 6-³H is higher in PP than in PV hepatocytes, is greater at low than at high glucose concentrations, and is greater in the absence than in the presence of insulin (Table 5). In guinea pig but not in pig hepatocytes, the effect of mercaptopicolinate on pyruvate cycling was dependent on the glucose concentration, as in the rat,

the percentage contribution of pyruvate recycling was greater in the absence than in the presence of insulin (Table 5). These estimates for pyruvate cycling in rat and guinea pig hepatocytes are relative to loss of 6-³H and do not parallel changes in absolute rates because of the effects of mercaptopicolinate on glycogen synthesis. However, in pig hepatocytes, in contrast with rat and guinea pig hepatocytes, there was negligible effect of mercaptopicolinate on the direct pathway (10 mM glucose: control, 9.5; mercaptopicolinate, 8.9; insulin, 43.8; insulin + mercaptopicolinate, 35.0 nmol of ³H incorporated/3 h per mg), and the estimates in Table 5 parallel the changes in rates of incorporation of 6-³H. It might be assumed, therefore, that the effects of mercaptopicolinate on the indirect pathway (¹⁴C-³H incorporation: control, 2.5; mercaptopicolinate, 0.09; insulin, 16.2; insulin + mercaptopicolinate, 3.0 nmol/3 h per mg of protein) are largely due to inhibition of phosphoenolpyruvate carboxykinase. Insulin increased the indirect pathway 6.4-fold (2.5 to 16.2 nmol/3 h per mg of protein) as compared with a 4.6-fold increase in the direct pathway (9.5 to 43.8 nmol/3 h per mg of protein). Pyruvate cycling, determined from the inhibition by mercaptopicolinate, accounted for 96% of the indirect pathway in the absence of insulin (control, 2.5; mercaptopicolinate, 0.09 nmol/3 h per mg of protein) as compared with 81% in the presence of insulin (insulin, 16.2; insulin + mercaptopicolinate, 3.0 nmol/3 h per mg of protein). From this, it can be estimated that 78% of the increase in the indirect pathway caused by insulin is mercaptopicolinate-sensitive, suggesting that a high proportion of the increased loss of 6-³H caused by insulin is due to pyruvate cycling.

DISCUSSION

We show in this study that the incorporation of [U-¹⁴C]glucose into glycogen correlates with glycogen deposition over a range of glucose concentrations (10–30 mM), in both the absence and the presence of insulin. It can be inferred, therefore, that the incorporation of [U-¹⁴C]glucose into glycogen represents the sum of the rates of glycogen synthesis from glucose by the direct and indirect pathways (involving substrate cycling). ³H in the 6-position of glucose is lost when [3-³H,U-¹⁴C]pyruvate formed from glucose is reconverted to glucose 6-phosphate [6,9,10]. The amount of 6-³H that is retained during conversion of pyruvate to glucose has been estimated to be about 8% (from the incorporation of [3-³H,3-¹⁴C]lactate into glycogen [6]). Consequently, loss of 6-³H during pyruvate cycling is assumed to be complete [6]. Theoretically, loss of 6-³H could occur by additional mechanisms that do not involve cycling via pyruvate [13], and it remains controversial whether these additional mechanisms account for a negligible [14] or significant [13] proportion of total loss of 6-³H.

In the present study we used mercaptopicolinate, an inhibitor of phosphoenolpyruvate carboxykinase [15–17], to determine the relative contribution of pyruvate cycling to the overall loss of 6-³H label during conversion of [6-³H,U-¹⁴C]glucose to glycogen in hepatocytes. The validity of this approach rests on two assumptions: first, that mercaptopicolinate blocks the conversion of pyruvate to glucose 6-phosphate; and secondly, that mercaptopicolinate does not affect the loss of ³H from the 6-position by mechanisms other than altering cycling via pyruvate. The similar percentage inhibition by mercaptopicolinate of [¹⁴C]pyruvate incorporation into both glycogen and glucose suggests that mercaptopicolinate inhibits pyruvate metabolism before formation of glucose 6-phosphate rather than by inhibition of glucose-6-phosphatase. The lack of effect of mercaptopicolinate on the ³H/¹⁴C ratio in glycogen during metabolism of [3-³H,U-

¹⁴C]glucose (Table 2) suggests that mercaptopicolinate may not affect loss of label at the triose phosphate level. However, whether mercaptopicolinate affects loss of 6-³H by other mechanisms cannot be rigorously excluded. It seems unlikely that the effects of mercaptopicolinate on the ³H/¹⁴C ratio in glycogen are due to cell swelling, since a hypo-osmolar medium which caused a similar increase in cell volume as mercaptopicolinate did not affect the ³H/¹⁴C ratio. We used a concentration of mercaptopicolinate (1 mM) that causes 89% inhibition of [¹⁴C]pyruvate incorporation into glucose. Higher concentrations of mercaptopicolinate did not cause further inhibition of gluconeogenesis from pyruvate (Figure 1a), although they caused progressive inhibition of glycogen synthesis (Figure 1b). The latter effect is probably independent of phosphoenolpyruvate carboxykinase, since it was associated with inhibition of incorporation of 6-³H into glycogen and presumably therefore of the direct pathway. The biphasic effect of mercaptopicolinate on glycogen synthesis is consistent with previous reports that 0.5–1 mM mercaptopicolinate stimulates glycogen synthesis in rat hepatocytes [24,25]. The stimulatory effect has been attributed to cell swelling [26] or to inhibition of glucose-6-phosphatase [27,28], but the cause of the inhibitory effect at high mercaptopicolinate concentrations (which is not associated with cell damage, as assessed from lack of leakage of lactate dehydrogenase) has not been identified. Our results demonstrate an increase in cell volume with mercaptopicolinate, but do not support a role for inhibition of glucose-6-phosphatase in the present experimental conditions. Whether the stimulation of glycogen synthesis from glucose by mercaptopicolinate (0.25–1 mM) is due to cell swelling remains to be established. Since changes in cell volume are frequently associated with changes in pH, which may have converse effects on glycogen synthesis and glycolysis [31], the metabolic effects of mercaptopicolinate may be due to changes in pH as well as cell volume.

If it is assumed that the increment in the ³H/¹⁴C ratio in glycogen caused by mercaptopicolinate represents the contribution of pyruvate cycling to loss of 6-³H, then the present study shows the following: first, that pyruvate cycling is greater in PP than in PV hepatocytes; secondly, that in rat and guinea pig hepatocytes the contribution of pyruvate cycling to loss of 6-³H is higher at low glucose concentrations, whereas in pig hepatocytes pyruvate cycling totally accounts for loss of 6-³H at all glucose concentrations in the absence of insulin; and thirdly, that in all species studied the relative contribution of pyruvate cycling to loss of 6-³H is higher in the absence than in the presence of insulin, suggesting that the increase in the loss of 6-³H caused by insulin represents in part a mechanism not involving pyruvate cycling.

Glucose cycling relative to glycolytic and gluconeogenic enzyme capacity

During incubation of hepatocytes with glucose (10–30 mM) in the absence of lactate and pyruvate, hepatocyte monolayers accumulate lactate in the medium (Table 2) and the accumulation is linear with time for 3 h [19]. Recycling of glucose by conversion to pyruvate and reversion to glucose 6-phosphate and then to glycogen (Tables 3 and 5) therefore occurs under conditions of net lactate production.

The greater effect of mercaptopicolinate on the ³H/¹⁴C ratio in PP than in PV hepatocytes is of interest because of the heterogeneity of gluconeogenic and glycolytic enzymes in these cell populations [32,33]. The recognition that the activities of gluconeogenic enzymes, including phosphoenolpyruvate

carboxykinase, are higher in the afferent (PP) zone of the liver acinus, while the glycolytic enzymes have higher activities in the efferent (PV) zone, led to the theory of metabolic zonation which postulated that conversion of dietary glucose to C_3 intermediates occurs in the efferent zone while their reconversion to glucose 6-phosphate and then to glycogen occurs in the afferent zone, after entry into the circulation and return to the liver via the hepatic artery [32]. Studies on isolated PP and PV hepatocytes have established that the former have a higher gluconeogenic flux than the latter when incubated with pyruvate or lactate [20,34,35], a similar glycolytic flux at low glucose concentrations [19,35] but a lower glycolytic flux at high glucose concentrations [19]. Intracellular glucose cycling via pyruvate within the hepatocyte requires both gluconeogenic and glycolytic capacity. The greater effect of mercaptopicolinate on PP hepatocytes, which have a high gluconeogenic relative to glycolytic capacity compared with PV cells, suggests that the gluconeogenic rather than the glycolytic capacity may be the main determinant of intracellular glucose cycling via pyruvate. The greater contribution of pyruvate cycling at low than at high glucose concentrations is further support that the gluconeogenic rather than the glycolytic capacity favours cycling. Despite the small effect of mercaptopicolinate on loss of $6\text{-}^3\text{H}$ in PV cells, loss of $6\text{-}^3\text{H}$ in absolute terms, was high, suggesting that in these cells loss of $6\text{-}^3\text{H}$ largely represents mechanisms not involving pyruvate cycling. The sensitivity of gluconeogenesis to inhibition by mercaptopicolinate was similar in PP and PV hepatocytes (results not shown), suggesting that the small response in PV cells was not due to a lower sensitivity to mercaptopicolinate, but to a lower contribution of pyruvate cycling to net loss of $6\text{-}^3\text{H}$.

Effects of insulin on glucose cycling

Very little information is available on the effects of insulin on the contributions of the direct and indirect pathways to hepatic glycogen synthesis either *in vivo* or *in vitro*. Studies *in vivo* cannot distinguish between direct effects of insulin on the liver as distinct from secondary changes in delivery of gluconeogenic precursors to the liver. The question of whether insulin has a direct role in the control of hepatic glycogen deposition has been the subject of much controversy [8,36,37]. Insulin has been shown to stimulate glycogen synthesis in hepatocyte suspensions in some studies [38,39] but not in others [8,36,40]. It was concluded from the latter studies that insulin has no direct role in the control of hepatic glycogen synthesis when present as the sole hormone [8,36,40]. In contrast with the conflicting results from freshly isolated hepatocyte suspensions, studies using hepatocyte monolayers have established that insulin stimulates glycogen synthesis from glucose and gluconeogenic precursors when present as the sole hormone [19,41,42]. Few studies, however, have examined the short-term effects of insulin on glucose cycling *in vitro* [43–45]. These studies used [$3\text{-}^3\text{H}$, ^{14}C] and [$2\text{-}^3\text{H}$, ^{14}C] glucose, which do not provide information on cycling as far as pyruvate. The present study demonstrates that insulin increases the loss of $6\text{-}^3\text{H}$ during stimulation of glycogen synthesis in hepatocytes from rat, guinea pig, pig and human liver. This effect of insulin was observed over a range of glucose concentrations. Since the contribution of pyruvate cycling to loss of $6\text{-}^3\text{H}$ was lower in the presence of insulin, the effect of insulin is, at least in part, due to a mechanism that is independent of pyruvate cycling. Although in relative terms the contribution of pyruvate cycling to loss of $6\text{-}^3\text{H}$ is diminished in the presence of

insulin, in absolute terms pyruvate cycling is a component of the increased loss of $6\text{-}^3\text{H}$ caused by insulin. This is particularly evident in pig hepatocytes, where pyruvate cycling is a major component of the increased loss of $6\text{-}^3\text{H}$ caused by insulin. The mechanism by which insulin increases glucose cycling during stimulation of glucose conversion to glycogen and its physiological significance warrant further study.

We thank the Medical Research Council (U.K.), the Juvenile Diabetes Foundation (International) and NOVO/Nordisk for financial support.

REFERENCES

- Landau, B. R. and Wahren, J. (1988) *FASEB J.* **2**, 2368–2375
- Watford, M. (1988) *Trends Biochem. Sci.* **13**, 329–330
- Kurland, I. K. and Pilkis, S. J. (1989) *FASEB J.* **3**, 2277–2281
- Youn, J. H. and Bergman, R. N. (1990) *Am. J. Physiol.* **258**, E899–E906
- Baker, N. (1977) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **36**, 253–258
- Scotfield, R. F., Kosugi, K., Schumann, W. C., Kumaran, K. and Landau, B. R. (1985) *J. Biol. Chem.* **260**, 8777–8782
- Lang, C. H., Bagby, G. J., Blakesley, H. L., Johnson, J. L. and Spitzer, J. J. (1986) *Am. J. Physiol.* **251**, E584–E590
- McGarry, J. D., Kuwajima, M., Newgard, C. B. and Foster, D. W. (1987) *Annu. Rev. Nutr.* **7**, 51–73
- Dunn, A., Katz, J., Golden, S. and Chenoweth, M. (1976) *Am. J. Physiol.* **230**, 1159–1162
- Katz, J. and Rognstad, R. (1976) *Curr. Top. Cell. Regul.* **10**, 237–289
- Spence, J. T. and Koudelka, A. P. (1985) *J. Biol. Chem.* **260**, 1521–1526
- Lahtela, J. T., Wals, P. A. and Katz, J. (1990) *Am. J. Physiol.* **259**, E389–E396
- Landau, B. R. (1991) *Am. J. Physiol.* **261**, E290–E291
- Katz, J. and Lahtela, J. (1991) *Am. J. Physiol.* **261**, E291–E292
- DiTullio, N. W., Berkoff, C. E., Blank, B., Kostos, V., Stack, E. J. and Saunders, H. L. (1974) *Biochem. J.* **138**, 387–394
- Kostos, V., DiTullio, N. W., Rush, J., Cieslinski, L. and Saunders, H. L. (1975) *Arch. Biochem. Biophys.* **171**, 459–465
- Jomain-Baum, M., Schramm, V. L. and Hanson, R. W. (1976) *J. Biol. Chem.* **251**, 37–44
- Al-Habori, M., Peak, M. and Agius, L. (1992) *Biochem. J.* **282**, 789–796
- Agius, L., Peak, M. and Alberti, K. G. M. M. (1990) *Biochem. J.* **266**, 91–102
- Tosh, D., Alberti, K. G. M. M. and Agius, L. (1988) *Biochem. J.* **256**, 197–204
- Agius, L., Peak, M. and Sherratt, H. S. A. (1991) *Biochem. Pharmacol.* **42**, 1711–1715
- Gerondaes, P., Alberti, K. G. M. M. and Agius, L. (1988) *Biochem. J.* **253**, 161–167
- Clifton, P. M., Chang, L. and McKinnon, A. M. (1988) *Anal. Biochem.* **172**, 165–168
- Bismut, H. and Plas, C. (1989) *Biochem. J.* **263**, 889–895
- Okajima, F. and Katz, J. (1979) *Biochem. Biophys. Res. Commun.* **87**, 155–162
- Baquet, A., Hue, L., Meijer, A. J., van Woerkom, G. M. and Plomp, P. J. A. M. (1990) *J. Biol. Chem.* **265**, 955–959
- Bode, A. M. and Nordlie, R. C. (1990) *FASEB J.* **4**, A710 (Abstract 2572)
- Bode, A. M., Nordlie, R. C. and Foster, J. D. (1992) *FASEB J.* **6**, A1482 (Abstract 3166)
- Goodman, M. N. (1975) *Biochem. J.* **150**, 137–139
- Clark, D., Lee, D., Rognstad, R. and Katz, J. (1975) *Biochem. Biophys. Res. Commun.* **67**, 212–219
- Peak, M., Al-Habori, M. and Agius, L. (1992) *Biochem. J.* **282**, 797–805
- Jungermann, K. and Katz, N. (1989) *Physiol. Rev.* **69**, 708–764
- Quistorff, B. (1990) *Essays Biochem.* **25**, 84–136
- Quistorff, B. (1985) *Biochem. J.* **229**, 221–226
- Chen, K. S. and Katz, J. (1988) *Biochem. J.* **255**, 99–104
- Katz, J. and McGarry, J. D. (1984) *J. Clin. Invest.* **74**, 1901–1909
- Hers, H. G. (1990) *J. Inher. Metab. Dis.* **7**, 51–73
- Nyffeller, F., Fasel, P. and Walter, P. (1981) *Biochim. Biophys. Acta* **675**, 17–23
- Beynen, A. C. and Geelen, M. J. H. (1981) *Ind. J. Biochem.* **19**, 46–48
- Boyd, M. E., Albright, E. R., Foster, D. W. and McGarry, J. D. (1981) *J. Clin. Invest.* **68**, 142–151
- Schudt, C. (1980) *Biochim. Biophys. Acta* **629**, 499–509
- Fleig, W. E., Enderle, D., Steudter, S., Nother-Fleig, G. and Ditschuneit, H. (1987) *J. Biol. Chem.* **262**, 1155–1160
- Parniak, M. and Kalant, N. (1985) *Can. J. Biochem. Cell Biol.* **63**, 333–340
- Parniak, M. and Kalant, N. (1988) *Biochem. J.* **251**, 795–802
- Salhanick, A. I., Chang, C. L. and Amatruda, J. M. (1989) *Biochem. J.* **261**, 985–992