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# Implications of the simultaneous occurrence of hepatic glycolysis from glucose and gluconeogenesis from glycerol

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Glycolysis from [6-$^3$H]glucose and gluconeogenesis from [U-$^{14}$C]glycerol were examined in isolated hepatocytes from fasted rats. A 5 mM bolus of glycerol inhibited phosphorylation of 40 mM glucose by 50% and glycolysis by more than 60%, and caused cellular ATP depletion and glycerol 3-phosphate accumulation. Gluconeogenesis from 5 mM glycerol was unaffected by the presence of 40 mM glucose. When nonsaturating concentrations of glycerol ($< 200$ μM) were maintained in the medium by infusion of glycerol, cellular ATP concentrations remained normal. The rate of uptake of infused glycerol was unaffected by 40 mM glucose, but carbohydrate synthesis from glycerol was inhibited 25%, a corresponding amount of glycerol being diverted to glycolytic products, whereas 10 mM glucose had no inhibitory effect on conversion of infused glycerol into carbohydrate. Glycerol infusion depressed glycolysis from 10 mM and 40 mM glucose by 15 and 25%, respectively; however, the overall rates of glycolysis were unchanged because of a concomitant increase in glycolysis from the infused glycerol. These studies show that exposure of hepatocytes to glucose and low quasi-steady-state concentrations of glycerol result in the simultaneous occurrence, at substantial rates, of glycolysis from glucose and gluconeogenesis from the added glycerol. We interpret our results as demonstrating that, in hepatocytes from normal rats, segments of the pathways of glycolysis from glucose and gluconeogenesis from glycerol are compartmentalized and that this segregation prevents substantial cross-over of phosphorylated intermediates from one pathway to the other. The competition between glucose and glycerol implies that glycolysis and phosphorylation of glycerol take place in the same cells, and that the occurrence of simultaneous glycolysis and gluconeogenesis may indicate channelling within the cytoplasm of individual hepatocytes.

*Keywords*: compartmentalization; gluconeogenesis; glycerol metabolism; glycolysis; metabolic channelling.

The mammalian liver has the capability for both glycolysis and gluconeogenesis. In the fed state, a major fate of glucose is glycolysis to pyruvate and lactate, which serve as precursors for lipid synthesis. In the fasted animal, in which hepatic lipogenesis is greatly diminished, metabolites such as lactate and glycerol, generated in the peripheral tissues, are taken up by the liver and converted into glucose. However, hepatocytes from fasted animals are also capable of substantial rates of glycolysis [1,2]. It is generally assumed that glycolysis and gluconeogenesis do not occur simultaneously in the same cell, but rather that metabolic conditions or allosteric effectors that stimulate flux along one pathway depress flow in the opposite direction. The actual direction of flow at any given moment is thought to be determined by regulatory mechanisms that control flux through the enzymatic steps specific to glycolysis and gluconeogenesis [3–5]. Moreover, evidence based on enzyme distribution in the liver suggests that metabolic zonation within the hepatic lobule exists, favouring gluconeogenesis in the periportal region [6].

Glycerol is an important gluconeogenic substrate, especially in the fasting state [7,8], and the bulk of the glycerol reaching the liver is converted into glucose [9]. The question therefore arises as to the fate of glycerol when glycolysis is induced in hepatocytes from fasting animals by a glucose load [2]. In this paper we report that, when isolated hepatocytes from fasted rats are incubated with glycerol and glucose in combination, glycolysis from glucose, and gluconeogenesis from glycerol, proceed simultaneously at substantial rates. The implications of these findings are discussed.

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*Abbreviations*: Fru-2,6-$P_2$, fructose 2,6-bisphosphate; Glc-6-*P*, glucose 6-phosphate; Gro-3-*P*, glycerol 3-phosphate; $S_{0.5}$, substrate concentration yielding half-maximal reaction rate.

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## MATERIALS AND METHODS

### Materials

Collagenase and enzymes necessary for the assay of metabolites were from Roche Diagnostics Australia (Castle Hill, NSW, Australia) as was BSA (fraction V), which was defatted as described by Chen [10]. Inulin was obtained from Sigma (St Louis, MO, USA) and inulinase (Novozym 230) was a gift from Novo Nordisk A/S (Bagsvaerd, Denmark). All other chemicals were of the highest purity commercially available. HPLC-purified [2-$^3$H]glucose and [6-$^3$H]glucose were obtained from New England Nuclear (Boston, MA, USA), and [U-$^{14}$C]glycerol from Amersham

Pharmacia Biotech (Castle Hill, NSW, Australia). Dowex AG50-X8 ($H^+$, 100–200 mesh) and Dowex AG1-X8 ($Cl^-$, 100–200 mesh), for the separation of radiolabelled glucose and its metabolic products, were obtained from Bio-Rad (Hercules, CA, USA).

### Preparation and incubation of hepatocytes

Hepatocytes were prepared from male Hooded Wistar rats (280–300 g body wt), starved for 24 h to deplete liver glycogen, by a modification [11] of the method of Berry & Friend [12], in which 1 mM $Ca^{2+}$ was added to the washing medium. The hepatocytes (≈ 100 mg wet wt) were incubated at 37 °C in 2 mL of a balanced bicarbonate–saline containing 2.25% (w/v) albumin, with a gas phase of 95% $O_2$/5% $CO_2$ [13,14]. The incubation mixtures initially contained 1 μCi [6-$^3$H]glucose for determination of the rate of glycolysis from glucose [2] and 1.0 μCi [2-$^3$H]glucose for determination of the rate of glucose phosphorylation [1]. For the measurement of glycerol metabolism, the incubation vessels were infused with 0.14 M [U-$^{14}$C]glycerol (specific radioactivity 48 000 d.p.m·μmol$^{-1}$) at a rate of 0.138 ± 0.006 μmol·min$^{-1}$. In experiments in which $CO_2$ generation was measured, duplicate incubations were carried out in sealed vials; perchloric acid was injected through the seal at the end of the incubation period, and $^{14}CO_2$ collected in phenylethylamine (0.25 mL) [15].

In a number of experiments, we employed 40 mM glucose because the substrate concentration yielding half-maximal reaction rate ($S_{0.5}$) for glucokinase is more than doubled *in vitro* [16]. We have previously observed that hepatocytes exposed to this substrate concentration carry out glycolysis at rates observed *in vivo* [3,15,17]. In other studies we used 10 mM glucose, together with trace amounts of fructose generated from inulin by inulinase [18]. This constant generation of fructose, which maintains a concentration of 70 μM in the medium, significantly lowers the *in vitro* $S_{0.5}$ of glucokinase for glucose [18], although not to the value seen *in vivo* [16,19]. The metabolism of the fructose formed from inulin did not contribute significantly to glucose formation [18]. To maintain nonsaturating concentrations of glycerol in the incubation medium, we infused glycerol by means of a high-precision infusion pump (Braun, Melsungen, Germany) adapted to hold an array of 24 1-mL tuberculin syringes (Becton Dickinson, Singapore). To avoid significant dilution of the incubation mixture, an infusion rate of 0.985 ± 0.005 μL·min$^{-1}$ ($n$ = 20) was selected.

### Analytical procedures

At the completion of the incubation period, a 0.5-mL sample was deproteinated with 1.5 mL ice-cold ethanol for the measurement of isotopic products of glucose and glycerol metabolism. Fructose 2,6-bisphosphate (Fru-2,6-$P_2$) was stabilized by mixing 0.3 mL of the contents of the incubation vessel with 0.3 mL 0.1 M NaOH and the mixture heated at 80 °C for 10 min [20]. Samples were stored at 4 °C until assayed. All extracts were diluted 10-fold with 10 mM NaOH before assay as described by Van Schaftingen *et al.* [20]. The remaining portion of the incubation mixture was deproteinated with an equal volume of ice-cold 1 M perchloric acid and neutralized before the metabolites were measured by standard enzymatic techniques [21]. In confirmatory experiments, the isotopic products of glucose and glycerol were also determined in the perchloric acid-precipitated neutralized medium, and results similar to those obtained with ethanol deproteination were obtained. Radiolabelled glucose and water were separated by ion-exchange chromatography [22,23]. The radiolabelled products of glycerol metabolism were also separated in this manner. The rate of glycolysis was determined from the sum of tritium from [6-$^3$H]glucose recovered in water, lactate, pyruvate and amino acids [1] and the rate of glucose phosphorylation from the sum of $^3H_2O$ released from [2-$^3$H]glucose plus the amount of tritiated glycogen formed [1]. In experiments in which 10 mM glucose was added, when the rates of glucose metabolism were calculated, allowance was made for the change in glucose specific radioactivity over the course of the incubation period [18]. Isotopic glycogen formation was measured as previously described [1]. Determination of the rate of glucose/glucose 6-phosphate (Glc-6-*P*) cycling was performed as described previously [15]. To simplify balance studies, the rates of glucose and glycerol metabolism are expressed as μmol $C_6$ equivalents·min$^{-1}$·(g wet weight)$^{-1}$ (mean ± SEM). Statistical analysis was carried out using Student's *t*-test for unpaired data.

## RESULTS

### Effects of a bolus of glycerol on hepatic carbohydrate metabolism

In initial studies, hepatocytes from fasted rats were incubated with 40 mM [6-$^3$H]glucose in the absence or presence of a bolus of 5 mM [$^{14}$C]glycerol. Under these conditions, there was no significant change in the rate of gluconeogenesis from glycerol in the presence of 40 mM glucose [0.65 ± 0.02 to 0.60 ± 0.03 μmol·min$^{-1}$·(g wet weight)$^{-1}$; $n$ = 5], whereas the glycolytic rate from glucose was inhibited by more than 60% [0.96 ± 0.03 to 0.33 ± 0.02 μmol·min$^{-1}$·(g wet weight)$^{-1}$ ($n$ = 5, $P$ < 0.001)] in the presence of glycerol. We also observed that, in hepatocyte suspensions exposed to glycerol, added as a bolus to achieve initial concentrations in the incubation medium of 0.5–5.0 mM, there was an immediate rise in both dihydroxyacetone phosphate and, in particular glycerol 3-phosphate (Gro-3-*P*), whereas ATP concentrations fell. The extent of these changes and the rate of glycerol uptake and glucose synthesis were dependent on the initial concentration of added substrate and were maximal by 5 mM (Table 1). Closely similar changes were observed when glycerol and glucose were added in combination. These effects of glycerol are apparently a consequence of the trapping of phosphate in phosphorylated intermediates and are analogous to those brought about by exposure of hepatocytes to high concentrations of fructose [24].

The generation of $^3H_2O$ from [2-$^3$H]glucose provides a good measure of the rate of hepatic phosphorylation of glucose *in vitro* [2,25]. Incubation of hepatocytes with [2-$^3$H]glucose (Table 2) showed that glucokinase activity was impaired by exposure of cells to a 5-mM bolus of glycerol so that rates of glucose phosphorylation were decreased by 47% ($P$ < 0.001). Duplicate experiments in which [6-$^3$H]glucose was substituted for [2-$^3$H]glucose were carried out to measure the effects of glycerol on glucose cycling through Glc-6-*P*. Glycerol addition significantly

**Table 1. Effect of initial glycerol concentration on rates of glycerol removal, glucose formation, and cellular concentrations of ATP, dihydroxyacetone phosphate (DHAP) and Gro-3-*P*.** Hepatocytes (100 mg wet wt) from fasted rats were incubated under standard conditions in the presence of initial glycerol concentrations of 0.5–5 mM. The cellular concentrations [μmol·(g wet wt)$^{-}$1] of ATP, DHAP and Gro-3-*P* were measured at 5, 10 or 20 min depending on the initial glycerol concentration and correspond to the maximum rate of glycerol removal for each initial glycerol concentration. Data are presented as the mean ± SEM ($n$ = 5). Glycerol uptake and glucose formation are expressed as μmol $C_6$ equivalents·min$^{-1}$·(g wet wt)$^{-1}$.

| [Glycerol] (mM) | [DHAP] | [Gro-3-*P*] | [ATP] | Glycerol uptake | Glucose formation |
|---|---|---|---|---|---|
| 0.5 | 0.09 ± 0.01 | 2.25 ± 0.09 | 2.14 ± 0.14 | 0.56 ± 0.04 | 041 ± 0.11 |
| 1.0 | 0.13 ± 0.01 | 3.93 ± 0.14 | 1.71 ± 0.06 | 0.78 ± 0.07 | 053 ± 0.02 |
| 2.0 | 0.20 ± 0.01 | 7.09 ± 0.24 | 1.29 ± 0.08 | 0.80 ± 0.02 | 072 ± 0.02 |
| 3.0 | 0.24 ± 0.02 | 7.89 ± 0.46 | 1.24 ± 0.05 | 0.92 ± 0.01 | 076 ± 0.02 |
| 4.0 | 0.24 ± 0.02 | 7.87 ± 0.39 | 0.98 ± 0.04 | 1.02 ± 0.03 | 085 ± 0.03 |
| 5.0 | 0.28 ± 0.02 | 8.44 ± 0.38 | 0.80 ± 0.03 | 0.98 ± 0.03 | 087 ± 0.04 |

decreased the rate of glucose utilization ($P$ < 0.001) and lowered the rate of cycling through Glc-6-*P* by 25% ($P$ < 0.05) (Table 2). However, under these conditions the proportion of glucose phosphorylated that was recycled back to glucose was increased from 40 to 60%. As with hepatocytes incubated in the absence of glucose (Table 1), the bolus addition of 5 mM glycerol resulted in an accumulation of intracellular Gro-3-*P* and depletion of ATP; the concentration of Fru-2,6-$P_2$ fell by over 90% (Table 2).

## Effect of glycerol infusion on hepatic carbohydrate metabolism

These initial studies indicated the desirability of maintaining low concentrations of glycerol in the incubation medium. Because this substrate is rapidly metabolized by hepatocytes, this required continuous infusion of the substrate at a nonsaturating rate. Preliminary experiments established that, when glycerol was infused at a rate of 0.138 ± 0.006 μmol·min$^{-1}$ ($n$ = 10), cellular ATP concentrations and near-maximal rates of glucose synthesis were maintained (Table 3). Under these conditions, there was a near-stoichiometric conversion of glycerol into glucose. Samples taken at 10-min intervals, over a period of 1 h under these conditions, showed that medium glycerol concentrations did not rise above 200 μM and intracellular Gro-3-*P* was consistently less than 1.5 mM. Higher rates of glycerol infusion resulted in the depletion of cellular ATP and accumulation of Gro-3-*P*, but had little effect on the rate of glucose synthesis.

These experiments on glucose–glycerol interactions were repeated by incubating hepatocytes with 40 mM [6-$^3$H]glucose, together with infusion of [$^{14}$C]glycerol. After an initial incubation period of 10 min, during which metabolic changes became linear, isotopic measurements taken over the subsequent 50 min, revealed that more than 90% of infused [U-$^{14}$C]glycerol was converted into glucose plus glycogen. Lactate and $CO_2$ formation were minimal, and no pyruvate was detected (Table 4). The rate of gluconeogenesis (glucose + glycogen) from [U-$^{14}$C]glycerol, infused when the incubation medium contained 40 mM [6-$^3$H]glucose, was about 25% less than that observed with glycerol alone ($P$ < 0.01), as measured by incorporation of [$^{14}$C]glycerol into glucose + glycogen, and substantial amounts of $^{14}$C were now detected in the lactate, pyruvate and $CO_2$. Moreover, when glycerol was infused with glucose present, glycolysis from glucose was inhibited by about 25% ($P$ < 0.001), but the overall rate of glycolysis was unchanged (Table 4).

We also examined the effects of glycerol infusion on carbohydrate metabolism when hepatocytes were incubated with 10 mM [6-$^3$H]glucose, inulin and inulinase (Fig. 1). When glycerol was infused, glucose accumulated in the medium at a rate of 0.25 ± 0.03 μmol·min$^{-1}$·(g wet weight)$^{-1}$ ($n$ = 5) whereas, in the absence of glycerol infusion, glucose was removed at 0.37 ± 0.02 μmol·min$^{-1}$·(g wet weight)$^{-1}$ ($n$ = 5). Thus in the presence of glycerol, there was an apparent net synthesis of glucose of 0.62 ± 0.05 μmol·min$^{-1}$·(g wet weight)$^{-1}$. The rate of glycogen synthesis of 0.13 ± 0.01 μmol·min$^{-1}$·(g wet

**Table 2. Effect of a bolus addition of glycerol on hepatic glucose metabolism.** Hepatocytes (100 mg wet wt) from fasted rats were incubated under standard conditions with 40 mM glucose in the absence and presence of 5 mM glycerol. The rates of glucose phosphorylation were measured as the sum of $^3H_2O$ released from [2-$^3$H]glucose plus the amount of tritiated glycogen formed. The rate of [6-$^3$H]glucose utilization represents the sum of tritium from [6-$^3$H]glucose recovered in water, lactate, pyruvate, amino acids and glycogen. The rate of Glc/Glc-6-*P* cycling was calculated from the difference between the rates of glucose phosphorylation and [6-$^3$H]glucose utilization [expressed as μmol $C_6$ equivalents·min$^{-1}$·(g wet wt)$^{-1}$]. The cellular concentrations of ATP and Gro-3-*P* [expressed as μmol·(g wet wt)$^{-1}$] and Fru-2,6-$P_2$ [expressed as nmol·(g wet wt)$^{-1}$] were measured after 30 min incubation. Data are presented as the mean ± SEM ($n$ = 5).

| Treatment | Glucose phosphorylation | [6-$^3$H]Glucose utilization | Glc/Glc-6-*P* cycling | [ATP] | [Gro-3-*P*] | [F2,6-P] |
|---|---|---|---|---|---|---|
| 40 mM Glucose | 1.95 ± 0.06 | 1.14 ± 0.07 | 0.81 ± 0.07 | 2.46 ± 0.04 | 0.47 ± 0.04 | 17.88 ± 0.07 |
| 40 mM Glucose + 5 mM glycerol | 1.03 ± 0.05[a] | 0.42 ± 0.03[a] | 0.61 ± 0.05[b] | 0.84 ± 0.03[a] | 8.89 ± 0.13[a] | 1.25 ± 0.10[a] |

[a,b]$P$ < 0.001 and $P$ < 0.01, respectively, for the effect of 5 mM glycerol addition.

**Table 3. Effect of glycerol metabolism on hepatocytes from fasted rats.** Hepatocytes from fasted rats were incubated either in the presence of an initial glycerol concentration of 5 mM or under conditions where glycerol was infused at 0.138 ± 0.006 μmol·min$^{-1}$. The cellular concentrations of ATP and Gro-3-*P* [μmol·(g wet wt$^{-1}$)] and Fru-2,6-$P_2$ [nmol·(g wet wt$^{-1}$)] were measured after 30 min incubation and the rates of glucose formation and glycerol removal [μmol $C_6$ equivalents·min$^{-1}$·(g wet wt$^{-1}$)] were determined between 10 and 30 min. Data are presented as the mean ± SEM ($n$ = 5).

| Treatment | Glucose formation | Glycerol utilization | [ATP] | [Gro-3-*P*] | [Fru-2,6-*P*] |
|---|---|---|---|---|---|
| Endogenous | – | – | 2.12 ± 0.04 | 0.29 ± 0.02 | 0.58 ± 0.03 |
| Glycerol added at 5 mM | 0.87 ± 0.03 | 0.98 ± 0.03 | 0.80 ± 0.03 | 8.44 ± 0.38 | 0.45 ± 0.04 |
| Glycerol infused at 0.138 ± 0.006 μmol·min$^{-1}$ | 0.59 ± 0.02 | 0.68 ± 0.01 | 2.24 ± 0.13 | 1.47 ± 0.11 | 2.54 ± 0.14 |

**Table 4. Metabolism of added glucose and infused glycerol separately and in combination.** Hepatocytes from fasted rats were incubated with either 40 mM glucose or 10 mM glucose, together with 0.12% (w/v) inulin and 10 mU inulinase, for periods of up to 60 min in the presence and absence of a glycerol infusion. Where indicated, glycerol was infused at 0.138 ± 0.006 μmol·min$^{-1}$ ($n$ = 10). The rate of glycolysis from glucose was measured with [6-$^3$H]glucose and determined from the sum of tritium recovered in water, lactate, pyruvate and amino acids. The rates of glycerol conversion into glucose, glycogen, lactate and pyruvate were determined by measuring incorporation of [$^{14}$C]glycerol into these products. The rate of glycolysis from glycerol was calculated from the sum of $^{14}$C-labelled lactate, pyruvate and $CO_2$. Metabolic rates are expressed as μmol $C_6$ equivalents·min$^{-1}$·(g wet wt$^{-1}$). The cellular concentration of Fru-2,6-$P_2$ [nmol·(g wet wt)$^{-1}$] was measured after 30 min incubation. Data are presented as the mean ± SEM ($n$ = 5).

| Treatments | Glucose metabolism (glycolysis) | Glycerol metabolism | | | | | [Fru-2,6-*P*] |
|---|---|---|---|---|---|---|---|
| | | Glucose | Glucose + glycogen | Lactate + pyruvate | Glycolysis | Glycerol utilization | |
| 40 mM Glucose | 0.96 ± 0.03 | – | – | – | – | – | 17.88 ± 0.07 |
| 40 mM Glucose + glycerol infusion | 0.73 ± 0.03[c] | 0.32 ± 0.02[a] | 0.41 ± 0.03[a] | 0.18 ± 0.01[a] | 0.23 ± 0.02[a] | 0.64 ± 0.05 | 13.49 ± 0.17[a,c] |
| Glycerol infusion | – | 0.48 ± 0.01 | 0.55 ± 0.02 | 0.02 ± 0.01 | 0.03 ± 0.01 | 0.58 ± 0.03 | 2.52 ± 0.14 |
| 10 mM Glucose | 0.45 ± 0.02 | – | – | – | – | – | 13.18 ± 0.26 |
| 10 mM Glucose + glycerol infusion | 0.38 ± 0.01[d] | 0.43 ± 0.01[b] | 0.52 ± 0.02 | 0.11 ± 0.01[a] | 0.13 ± 0.02[a] | 0.65 ± 0.03 | 11.29 ± 0.33[a,e] |

[a,b] $P < 0.001$ and $P < 0.05$, respectively, for the effect of glucose on glycerol metabolism; [c] $P < 0.001$ for the effect of glycerol infusion on 40 mM glucose metabolism; [d,e] $P < 0.01$ and P < 0.001, respectively, for the effect of glycerol infusion on 10 mM glucose metabolism.

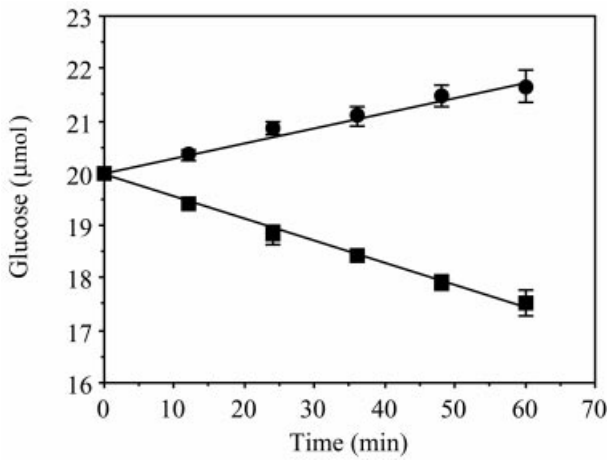


**Fig. 1. Effect of glycerol infusion on the glucose concentration in the incubation medium**. Hepatocytes (100 mg wet weight) from fasted rats were incubated in a total volume of 2 mL with 10 mM glucose plus 0.12% (w/v) inulin and 10 mU inulinase either alone (■) or together with an infusion of glycerol at 0.138 ± 0.006 μmol·min$^{-1}$ (●) for periods up to 60 min. The figure shows the change in the amount of glucose in the incubation medium, and data are presented as mean ± SEM ($n$ = 5).

weight)$^{-1}$ ($n$ = 5) was unaffected by the glycerol infusion. The basis for the effects of glycerol infusion is revealed by the isotopic data (Table 4). These show that when glycerol was infused into the medium, the rate of glycolysis was reduced by 20% ($P < 0.01$) even though the rate of glucose phosphorylation in the presence of glucose alone [0.73 ± 0.01 μmol $C_6$ equivalents·min$^{-1}$·(g wet weight)$^{-1}$, $n$ = 3] was not altered during the glycerol infusion [0.70 ± 0.02 μmol $C_6$ equivalents·min$^{-1}$·(g wet weight)$^{-1}$, $n$ = 3]. As gluconeogenesis from [U-$^{14}$C]glycerol occurred at a rate of 0.48 ± 0.01 μmol·min$^{-1}$·(g wet weight)$^{-1}$ ($n$ = 5), a net accumulation of carbohydrate took place. As with the incubations containing 40 mM glucose (Table 4), the overall rate of glycolysis was not significantly changed. The infusion of glycerol into hepatocytes incubated with 10 mM and 40 mM glucose lowered the cellular Fru-2,6-$P_2$ concentration by 15% and 25%, respectively (Table 4). This is in marked contrast with the effect of a bolus addition of 5 mM glycerol (Table 2) where a > 90% reduction in Fru-2,6-$P_2$ was measured. It was noteworthy that at both glucose concentrations, the percentage fall in cellular Fru-2,6-$P_2$ concentration resulting from glycerol infusion was equivalent to the percentage decrease in the rates of glycolysis. The fivefold rise in cellular Fru-2,6-$P_2$ concentration associated with the addition of glucose to

hepatocyte incubations infused with glycerol had a minimal effect on the rate of glucose + glycogen formation from glycerol (Table 4).

## DISCUSSION

In the experiments reported here, we used an infusion technique to maintain concentrations of glycerol below 200 μM in the incubation medium. Most experiments were conducted with 40 mM glucose in order to achieve near maximal flux through glucokinase. Moreover, the large glucose pool gave the advantage of reducing the likelihood of glucose, newly formed from glycerol, being subsequently glycolysed. In the absence of added glucose, about 90% of the glycerol taken up was converted into carbohydrate (glucose plus glycogen) and the balance was glycolysed. The rate of glycerol uptake was unaffected in the presence of 40 mM glucose, but carbohydrate synthesis from glycerol was inhibited 25%, a corresponding amount of glycerol being diverted to glycolytic products. However, the presence of 10 mM glucose had no significant inhibitory effect on glycerol conversion into carbohydrate. These findings can be explained on the basis that some of the glycolytic products generated from 40 mM glucose are recycled to glucose and glycogen [3] and can compete to some extent with gluconeogenesis from glycerol. This competition is overcome when glycerol is added as a bolus at saturating concentrations. Glycolytic products from glucose, added at 10 mM, are apparently recycled to a much lesser extent [3,26], and do not affect the rate of gluconeogenesis from infused glycerol.

The addition of a bolus of glycerol to hepatocytes incubated with 40 mM glucose inhibited glycolysis more than 60%. However, glycerol infusion depressed glycolysis from 40 mM glucose by only about 25%, and the overall rate of lactate + pyruvate formation (from glucose and glycerol) was unchanged because of a concomitant increase in the formation of glycolytic product from the infused glycerol. Glycerol infusion depressed glycolysis from 10 mM glucose by 20% and, under these conditions, about 17% of the glycerol carbon was diverted to glycolytic products. Glycerol appears to inhibit glycolysis from glucose by two mechanisms. When added as a bolus, it depresses glucose phosphorylation, presumably as the result of depletion of ATP. Under these conditions, there was a decrease in the rate of glucose recycling through Glc-6-*P*; however, the proportion of glucose phosphorylated recycled back to glucose was increased. When infused at a rate that maintains a glycerol concentration in the incubation medium below 200 μM, ATP was not depleted. It is difficult to reconcile the changes in cellular Fru-2,6-$P_2$ concentration resulting from glycerol infusion with the simultaneous rates of glycolysis from glucose and gluconeogenesis from glycerol. The inhibition of glycolysis is consistent with a lowering of the Fru-2,6-$P_2$ concentration and an inhibition of phosphofructokinase-2, but the rate of gluconeogenesis was unaltered in the presence of 10 mM glucose.

When glycerol was the only added substrate, more than 90% of the $^{14}C$ was recovered in glucose and glycogen and about 5% in glycolytic products. However, when 40 mM glucose was also present, the percentage of glycerol $^{14}C$ converted into glucose fell to about 65%, and 35% accumulated as glycolytic products. It can be envisaged that the operation of a redox couple between Gro-3-*P* and pyruvate, generated during glycolysis from glucose, facilitates the entry of some dihydroxyacetone phosphate and glyceraldehyde 3-phosphate, derived from glycerol, into the glycolytic pathway. This could take place by means of the interaction of cytoplasmic NAD-linked Gro-3-*P* and lactate dehydrogenases.

Our data, derived both from balance studies and isotopic experiments, show that exposure of hepatocytes to glucose and low quasi-steady-state concentrations of glycerol resulted in the simultaneous occurrence of glycolysis from glucose and gluconeogenesis from the added glycerol. The rate of carbohydrate synthesis from glycerol was ≈ 60% of the rate of glycolysis from 40 mM glucose and exceeded that of glycolysis from 10 mM glucose. The shared enzymes in the metabolic sequences from glucose to lactate and from glycerol to glucose are phosphohexose isomerase, aldolase and triose phosphate isomerase. These cytoplasmic enzymes are considered to catalyse reactions reversible in the presence of metabolite concentrations found intracellularly. The enzymes all have high activity in liver and are thought to keep the mass–action ratio of their substrates close to equilibrium [3].The conventional view is that the substrate pools of these enzymes are each considered to exist within a single aqueous and homogeneous cellular compartment, frequently referred to as the 'cytosol' [27]. In such a compartment, the fate of a triose phosphate molecule, expressed in terms of entry into the glycolytic or gluconeogenic pathway, should in no way be influenced whether its origin is exogenous glycerol or fructose 1,6-bisphosphate derived from glucose. Yet when hepatocytes were exposed to glycerol alone, over 90% of the substrate was converted into glucose. Moreover, even in a glycolysing environment, induced by the presence of 40 mM glucose, almost three times as much glycerol carbon entered the gluconeogenic pathway than formed glycolytic products. When the initial glucose concentration was set at 10 mM, which generated a rate of glycolysis about half of that observed with 40 mM glucose, less than one glycerol molecule in seven entered the glycolytic pathway. These results do not seem compatible with the existence of a single homogeneous pool of triose phosphate contained within one cellular compartment. Rather it seems likely that the glycolytic and gluconeogenic fluxes that take place as a consequence of exposing hepatocytes to the substrate combination of glycerol and glucose reflect metabolic flows occurring in two separate cellular compartments, i.e. metabolic channelling.

We therefore interpret our results as demonstrating that, in hepatocytes from normal rats, segments of the pathways of glycolysis from glucose and gluconeogenesis from glycerol are compartmentalized and that this segregation prevents a substantial cross-over of phosphorylated intermediates from one pathway to the other. Brunengraber and coworkers have concluded from mass isotopomer distribution analysis that triose phosphate pools are not equally labelled by [$^{13}C$]glycerol in whole liver or isolated hepatocytes [28]. Malaisse *et al.* [29] have more recently made similar observations. This unequal labelling has been explained on the basis of the existence of different cell populations [28]. This possibility has not been conclusively

excluded in this study, in that our findings can be accounted for on the basis that the isolated cell preparation contains two types of hepatocyte, one kind with glycolytic and the other with gluconeogenic properties [6]. However, this seems improbable as the distribution of glycerokinase activity is approximately equal in periportal and perivenous hepatocytes [28]. Furthermore, there is considerable overlap in the distribution of the specific enzymes of glycolysis and gluconeogenesis in the hepatocyte lobule [6]. Thus, it seems likely that the irregular labelling of triose phosphates by [$^{14}$C]glycerol, described in [28], may reflect labelled and unlabelled forms of these metabolites coexisting in the same cell as a consequence of channelling. More direct evidence for this comes from our findings that there is competition between glycerol and glucose for the glycolytic pathway, and that glycolysis is impaired by high concentrations of Gro-3-*P*. Moreover, glycerol depresses glucose phosphorylation. As hepatocytes are generally impermeable to phosphorylated metabolites such as Gro-3-*P*, our observations suggest that glycolysis and phosphorylation of glycerol take place in the same cells, and that the occurrence of simultaneous glycolysis and gluconeogenesis is an indication of channelling within the hepatocyte cytoplasm of individual hepatocytes. Further studies to test this hypothesis are in progress.

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