# GLUCONEOGENESIS FROM L-LACTATE-2-T-2-$C^{14}$ IN PERFUSED RAT LIVER

G.MÜLLHOFER, O.KUNTZEN, S.HESSE and Th.BÜCHER

*Institut für Physiologische Chemie und Physikalische Biochemie der Universität München, München, Germany*

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## 1. Introduction

Problems in the understanding of cellular metabolism arise from the distribution of enzymes, cofactors and metabolites in different compartments separated by permeability barriers. The different localization of two key enzymes in the gluconeogenic pathway, i.e. mitochondrial pyruvate carboxylase (EC 6.4.1.1) and extramitochondrial phosphoenolpyruvate carboxykinase (EC 4.1.1.32) in rat liver [1–4], and the limited permeation of oxaloacetate through the mitochondrial membrane [5,6] has led to the postulation of shuttle metabolites for oxaloacetate transfer, such as aspartate [5] and glutamate [7]. Furthermore, a compartmentation of phosphorylated intermediates was discussed recently [8]. In order to identify the direct intermediate metabolites in glucose formation from lactate, livers of fasted rats were perfused with L-lactate-2-T-2-$C^{14}$. The specific tritium and carbon-14 activities of metabolites were determined and compared with the corresponding activities of glucose formed.

## 2. Materials and methods

Materials and general techniques have been described in the preceding paper [13]. In order to reduce the dilution of labeled glucose formed from lactate by unlabeled glucose originating from residual glycogenolysis, the liver was perfused first in a recirculating system without exogenous substrate for 30 min. Subsequently, the liver was perfused for 10 min in an open system with fresh solution, prior to the final perfusion with L-lactate-2-T-2-$C^{14}$ (5–6 mM). A freeze stop was made 11 min after onset of perfusion with labeled lactate.

Glucose was isolated from the perfusate leaving the liver immediately before freeze stop (precipitation of dextrane with methanol and deionization with Dowex-1X8-carbonate and Dowex-50-$H^+$) and was degradated by enzymatic and chemical procedures in 5 steps:

(1) Phosphorylation of glucose by hexokinase to glucose-6-P;

(2) Conversion of glucose-6-P to fructose-1, 6-di-P with phosphofructokinase;

(3) Splitting of fructose-1,6-di-P by aldolase into dihydroxyacetone-3-P and glyceraldehyde-3-P; the latter was trapped as 3-P-glycerate by the presence of glyceraldehyde-3-P dehydrogenase and arsenate in the incubation mixture. Tritium of NADH formed in this reaction was transferred to glutamate by adding α-ketoglutarate, ammoniumchloride and glutamate dehydrogenase;

(4) Dephosphorylation of 3-P-glycerate by acid phosphatase and splitting of glyceric acid by periodate in acid solution to glyoxylate and carbondioxide;

(5) Degradation of glyoxylate to carbondioxide and formate by hydrogen peroxide in neutral solution. All intermediates were purified by anion exchange chromatography; their specific tritium and carbon-14 activities were determined (table 1).

## 3. Results and discussion

A typical experiment is described in fig. 1. Following the addition of lactate, an increase in oxygen uptake was observed. The glucose production reached a

Table 1
Specific tritium and carbon-14 activities of derivatives formed from glucose, in relation to the specific activity of the lactate entering the liver. Specific activities of lactate: 0.785 $10^6$ cpm T/μmol; 0.302 $10^5$ cpm $C^{14}$/μmol.

| Derivatives | Including carbon atoms from position | Including hydrogen atoms from position | Relative tritium specific activity | Relative carbon-14 specific activity |
|---|---|---|---|---|
| Glucose | 1,2,3,4,5,6 | 1,2,3,4,5,6,6′ | 0.50 | 1.42 |
| Glucose-6-P | 1,2,3,4,5,6 | 1,2,3,4,5,6,6′ | 0.50 | 1.42 |
| Fructose-1,6-di-P | 1,2,3,4,5,6 | 1,1′,3,4,5,6,6′ | 0.47 | 1.45 |
| Dihydroxyacetone-3-P | 1,2,3 | 1,3 | 0.02 | 0.69 |
| 3-P-glycerate | 4,5,6 | 5,6,6′ | 0.14 | 0.65 |
| Glutamate | – | 4 | 0.26 | – |
| Glyoxylate | 4,5 | 5 | 0.00 | 0.34 |
| Formate | 5 | 5 | 0.00 | 0.29 |
| HOT* | – | 3,4,5 | 0.29 | – |

* From exchange of fructose-1,6-di-P with aldolase and triose-P-isomerase.

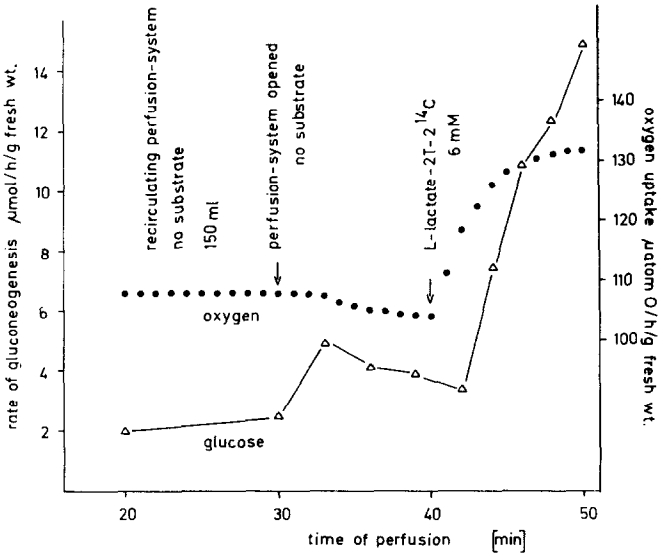


Fig. 1. Oxygen uptake and rate of gluconeogenesis of the perfused liver in relation to the time of perfusion. Perfusion temperature 33°C. Rat weight 305 g, fasted 48 hr before use, liver weight after freeze stop 16.5 g = fresh weight = 3.1 g dry weight.

rate of 15 μmoles/h/g fresh wt after 11 min. The extra oxygen consumed corresponded approximately to the energy required for enhanced gluconeogenesis, i.e. 1 μmol $O_2$ per 1 μmol glucose formed from lactate [9,10]. In table 2 the metabolite levels in the perfusate together with the total tritium and carbon-14 activities of the medium leaving the liver and the tritium incorporation into the water are shown.

### 3.1. *Metabolites of the gluconeogenic pathway*

The relative specific activities of perfusate glucose and tissue metabolites are summarized in table 3. With respect to carbon-14 activities, the metabolites may be divided into two groups. Lactate, malate, citrate and triose unit of glucose have almost the same relative specific activity as perfusate lactate. On the other hand, the relative specific activities of aspartate, glutamate and glycerol-3-P are considerably lower than that of lactate. Thus, it is evident from these data that malate is involved in the flux of carbon from lactate to glucose, whereas the participation of glutamata and aspartate appears to be problematic. The additional assumption of further compartmentation would be necessary in order to explain their minor relative specific activities. Although citrate shows an activity as high as malate its role as a direct intermediate in gluconeogenesis from lactate is doubtful [11].

The tritium activity of malate is equally distributed between positions 2 and 3 by the action of fumarase. It is assumed, therefore, that the carbon-14 label is also equilibrated. In fact, the positions 5 and 6 of glucose, originating from positions 2 and 3 of malate, show identical carbon-14 activities. An additional randomisation of carbon-14 into positions 1 and 4 of C4-dicarboxylic acids has to be considered due to the citric acid cycle flux. The extent of this type of randomisation depends on the ratio between the turn-

Table 2

Metabolite and radioactivity measurements in samples taken at different times from the medium leaving the liver. Lactate concentration in the medium entering the liver from 40 to 50 min of perfusion: 6.12 mM. Radioactivity: 4.82 $10^6$ cpm T/ml; 0.185 $10^6$ cpm $C^{14}$/ml. Perfusion temperature 33°C, flow rate 26 ml/min.

| Minutes of perfusion | Perfusion system | Glucose mM | Lactate mM | Pyruvate mM | Acetoacetate mM | Radioactivity cpm/ml X $10^{-6}$ Tritium | Carbon-14 | Tritium in HOH cpm/ml X $10^{-6}$ |
|---|---|---|---|---|---|---|---|---|
| 11 | Recirculating | 0.109 | – | – | – | | | |
| 20 | system 150 ml, | 0.142 | – | – | 0.44 | | | |
| 30 | no exogenous substrate | 0.183 | 0.08 | 0.02 | 0.59 | | | |
| 33 | Open system, | 0.052 | – | – | 0.19 | | | |
| 36 | no exogenous | 0.043 | – | – | 0.14 | | | |
| 39 | substrate | 0.041 | – | – | 0.23 | | | |
| 42 | Open system | 0.036 | 4.80 | 0.03 | 0.06 | 3.90 | 0.146 | 0.15 |
| 44 | L-lactate-2- | 0.078 | 5.24 | 0.08 | 0.09 | 4.36 | 0.164 | 0.33 |
| 46 | T-2-$C^{14}$ | 0.114 | 5.93 | 0.12 | 0.06 | 4.77 | 0.181 | 0.42 |
| 48 | | 0.130 | 5.93 | 0.12 | 0.09 | 4.78 | 0.172 | 0.45 |
| 50 | | 0.158 | 5.80 | 0.12 | 0.03 | 4.79 | 0.173 | 0.46 |

* β-hydroxybutyrate concentration < 0.02 mM.

Table 3

Specific tritium- and carbon-14 activities of metabolites isolated from the liver tissue. All specific activities are expressed in relation to the specific activities of the L-lactate-2-T-2-$C^{14}$ entering the liver.

| Metabolite | | Relative specific tritium activity | Relative specific carbon-14 activity | |
|---|---|---|---|---|
| Glutamate* | | 0.01 | 0.28 | |
| Aspartate* | | 0.09 | 0.13 | |
| Lactate | entering liver | 1.00 | 1.00 | |
| | leaving liver | 0.84 | 0.93 | |
| | liver tissue | 0.81 | 0.83 | |
| Malate* | total | 0.55 | 0.83 | |
| | 2 position | 0.27 | – | |
| | 3 position | 0.25 | – | |
| Citrate* | | 0.20 | 0.91 | |
| Glycerol-3-P* | total | 0.35 | 0.23 | |
| | 1 position | 0.05 | – | |
| | 2 position | 0.30 | – | |
| | 3 position** | 0.00 | – | |
| Glucose*** | total | 0.50 | 1.42 | |
| | 1 position | 0.00 | | |
| | 2 position | 0.03 | 0.69 | |
| | 3 position | 0.02 | | |
| | 4 position | 0.26 | | 0.05 |
| | 5 position | 0.00 | 0.65 | 0.29 |
| | 6 position | 0.14 | | 0.30 |

* Isolated from the tissue extract.

** By difference (total minus 1 plus 2 position).

*** Isolated from last perfusion fluid, see table 1 and methods.

over rate of oxaloacetate in the citric acid cycle and the rate of formation and decarboxylation of oxaloacetate. The low activity in position 4 of glucose indicates that this randomisation occurs at a small rate. Part of the randomized activity is lost as carbon dioxide by decarboxylation of oxaloacetate to phosphoenol-pyruvate. This may explain the small difference between the measured glucose carbon-14 activity (1.42) and the value of twice the malate activity (1.66). A dilution of labeled glucose for glucose formation not involving dicarboxylic acids may also contribute to the observed difference.

Considerable tritium activities were found only in positions 4 and 6 of glucose. The origin of this labeling pattern has been discussed in detail [12]. The activity in position 4 is identical with that of positions 2 and 3 of malate and position 2 of glycerol-3-P reflecting equilibration with the NAD/NADH pool [13]. On the other hand, the tritium activity in position 6 of glucose was about half of that in the corresponding position 3 of malate. Loss of tritium activity from position 6 of glucose may occur by interaction of the glycolytic with the gluconeogenic pathway. Glucose-6-P labeled only in positions 4 and 6 with tritium is reconverted to fructose-1,6-di-P and triosephosphates. The triosephosphates recondense after equilibration by triose-P-isomerase. The tritium activity of position 4 of fructose-1,6-di-P is constant, if fast equilibration of glyceraldehyde-3-P with the NADH pool is assumed.

3.2. *Compartmentation of the triosephosphate pool*

Triosephosphates are not directly accessible by our techniques, due to their low tissue levels. Their isotope distribution, however, can be derived from glucose and glycerol-3-P. The relative specific carbon-14 activity of the triosephosphates being the precursors of glucose should be half the specific activity of the symmetrically labeled glucose, i.e. 0.65–0.69. This value differs from the carbon-14 activity of 0.23 found in glycerol-3-P. On the other hand, tritium activity of position 2 of glycerol-3-P is found to be equilibrated with the NAD/NADH pool [13]. It must be assumed, therefore, that the dihydroxyacetone-3-P forming glucose belongs to a different pool as that equilibrated with glycerol-3-P via glycerol-3-P dehydrogenase. The missing tritium labeling of position 3 of glycerol-3-P presents further evidence for this compartmentation of triosephosphates. Moreover, this concept is supported by the low tritium activity of position 1 of glycerol-3-P compared to the labeling of position 4 of glucose (equilibrated with the NADH pool) in the presence of a high activity of triose-P-isomerase.

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**References**

[1] M.F.Utter and D.B.Keech, J. Biol. Chem. 238 (1963) 2603.
[2] I.Böttger , O.Wieland, D.Brdiczka and D.Pette, European J. Biochem. 8 (1969) 113.
[3] M.F.Utter and K.Kurahashi, J. Biol. Chem. 207 (1954) 821.
[4] C.Scrutton and M.F.Utter, Ann. Rev. Biochem. (1968) p. 270.
[5] H.A.Lardy, V.Paetkau and P.Walter, Proc. Nat. Acad. Sci. 53 (1965) 1410.
[6] J.M.Haslam and H.A.Krebs, Biochem. J. 107 (1968) 659.
[7] H.A.Lardy, E.Shrago, J.Young and V.Paetkau, Science 144 (1964) 564.
[8] C.J.Threlfall and D.F.Heath, Biochem. J. 110 (1968) 303.
[9] R.Scholz, J.Zehner and Th.Bücher, Acta Hepato-splenolog. 13 (1966) 376.
[10] R.Hems, B.D.Ross, M.N.Berry and H.A.Krebs, Biochem. J. 101 (1966) 284.
[11] R.A.Felicioli and R.Barsacchi, Ital. J. Biochem. 16 (1967) 331.
[12] H.D.Hoberman, J. Biol. Chem. 233 (1958) 1045.
[13] G.Müllhofer, O.Kuntzen, S.Hesse and Th.Bücher, FEBS Letters 4 (1969) 47.