Non-steady State Model Applicable to NMR Studies for Calculating Flux Rates in Glycolysis, Gluconeogenesis, and Citric Acid Cycle*

(Received for publication, June 2, 1994)

Guy Martiné, Marie-France Chauvin, Sylvie Dugelay, and Gabriel Baverel
From the Centre d’Etudes Métaboliques par Spectroscopie de Résonance Magnétique (CNRS EP 18), Hôpital Edouard Herriot, 69374 Lyon cedex 03, France

We present a mathematical model for calculating most reaction rates of glycolysis, gluconeogenesis and citric acid cycle in mammalian cells. The model also includes cycles such as the "phosphoenolpyruvate (PEP) → pyruvate → oxaloacetate → PEP" cycle and the "pyruvate → acetyl-CoA → citrate → citric acid cycle → oxaloacetate → PEP → pyruvate" cycle. The model, which does not require steady state conditions, is based on a set of equations, each one describing the fate of a given carbon of a selected intermediate. These fates are expressed as ratios of integrated transfer of this carbon to corresponding carbons in subsequent metabolites. At each bifurcation, the sum of all proportions adds up to 1. Among several calculation routes to determine a proportion value, we chose the one that was based on the most reliable parameter determined experimentally. The data introduced in the model are the micrometers of atom of traced carbon measured on each carbon of a number of products (corrected for natural tracer abundance). These incorporations can be measured by 13C NMR, gas chromatography-mass spectroscopy, or 14C counting.

Thanks to its flexibility, this model can be applied to data obtained with substrates other than glucose under many experimental conditions.

In the accompanying paper (12), we have conducted a study on glucose metabolism in isolated rabbit kidney tubules. For the interpretation of the data obtained, we have constructed a mathematical model that is based on the incorporation of 13C and 14C into various metabolites and allows calculation of most reaction rates of glycolysis, gluconeogenesis, and citric acid cycle in mammalian cells. This model, which is applicable to data obtained by 13C NMR, gas chromatography-mass spectroscopy, or 14C counting, is described in the present paper.

THEORY

Schematic Representation of Glucose Metabolism—A general representation of glucose metabolism is given in Fig. 1. This figure shows the main products accumulated during glucose metabolism, as well as the existence of different metabolic cycles that are functioning simultaneously and whose common metabolite is oxaloacetate.

In our model, the calculation of parameters (corresponding to ratios) is based on the fates of individual carbons 2, 1, and 3 of the glucose molecule, which are represented in Fig. 2.

* This work was supported in part by Grants CS 22-1-90 (Code 7) and CA 8-4-90 from the AFM. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed: Centre d’Etudes Métaboliques par Spectroscopie de Résonance Magnétique, Pavillon P, Hôpital Édouard Herriot, place d’Arsonval, 69374 Lyon cedex 03, France. Tel.: 33-78-77-86-85; Fax: 33-78-77-87-39.

† The abbreviations used are: Pyr, pyruvate; Glc, glucose; GroP, glycerol 3-phosphate; Gro, glycerol; PEP, phosphoenolpyruvate; Lac, lactate; AcCoA, acetyl coenzyme A; Glx, glutamate + glutamine; OAA, oxaloacetate; MET, metabolite.
Fig. 2. Metabolic fate of the C-3, C-2, and C-1 of glucose in rabbit kidney tubules. This figure shows the metabolic fate of glucose labeled either on its carbon 3, 2, or 1 which, for sake of simplicity, is represented as 3,2,1 GLC. Glucose metabolites are represented as α, β, and γ MET. Where MET represents any glucose-derived metabolite and α, β, and γ the labeled carbon of these metabolites when the labeled carbon of the glucose added as substrate was 3, 2, or 1, respectively. Unlabeled carbons of glucose metabolites are represented by a − sign. The amount (in pmol/h) of labeled glucose utilized is represented by X. Lowercase letters (or numbers) indicate the proportion of a given intermediate metabolized at a given step. A proportion represents the relative amount of a given intermediate that is converted into the next one. The fate of pyruvate derived from oxaloacetate, which is not shown, is identical to the fate of pyruvate formed by glycolysis. The oxaloacetate recycled after one complete Krebs cycle turn remains labeled when the substrate glucose is labeled on its carbon 2 or 1. Therefore, the fate of C-2 and C-1 of glucose requires more than one Krebs cycle turn to be defined. The synthesis of oxaloacetate resulting from the first Krebs cycle turn is considered to represent the beginning of the second turn. The relative amount of substrate (labeled glucose) transformed into any labeled intermediate or end product is obtained by multiplying the successive proportions found in the pathway from the substrate to the intermediate or end product of interest. The amount (named flux) expressed in C3 units of intermediate formed or end product accumulated during the incubation period (1 h) is obtained by multiplying the corresponding relative amount by twice the amount (X) of labeled glucose utilized. It is assumed that the proportion Σ of the oxaloacetate formed by the pyruvate carboxylase reaction equilibrates with fumarate, half of this oxaloacetate, equal to i, gives rise to oxaloacetate molecules having a symmetrical (inverted) labeling pattern.

Notations—Let us call \( [\text{C,MET}_{i,z}^{\text{C,Gluc}}] \) the amount of the metabolite (MET) labeled on its carbon y (where 1 ≤ y ≤ 6) arising from glucose labeled on its carbon x, where x is equal to 1, 2, or 3 because we used \([1^{-13}C]\text{glucose}, [2^{-13}C]\text{glucose}, \text{and} [3,4^{-13}C]\text{glucose}\) as labeled substrates and also because it is assumed that the C-4 and C-3 of glucose had the same metabolic fate.

Similarly, \( [\text{C}_{i,y}^{\text{C,MET}}]^\text{C,Gluc} \) is the amount of the metabolite (MET) labeled on its carbon y plus the amount of the metabolite (MET) labeled on its carbon y'; let us call \( [\text{C}_{i,y}^{\text{C,MET}}]^\text{C,Gluc} \) the amount of the metabolite labeled simultaneously on its carbons y and y';

Let us call \( \text{MET}_{i} \) the total amount of metabolite formed as a result of glucose metabolism.

Calculations of the (Parameters of the Model)—The different labels employed in our model to characterize parameters are defined in Table I.

The amount (in pmol/h) of any given intermediate or end product formed from the substrate (glucose) can be calculated by multiplying the amount of the substrate removed (X) by the successive proportions of intermediates passing through the different pathways leading to the intermediate or end product of interest.

Let us call X the sum of the glucose removal measured enzymatically plus the net formation of glucose via gluconeogenesis, noted \( \text{Glc}^{\text{Glc}} \).

Thus

\[
X = \text{Glc removed} + [\text{Glc}]^\text{Glc}
\]  
(Eq. 1)
Model of Glycolysis, Gluconeogenesis, and Citric Acid Cycle

An initial reasonable estimate of [Glc]$^{\text{Glc}}$ is introduced in Equation 1; successive adjustments are then made by comparison with the calculated value of [Glc]$^{\text{Glc}}$ until the proper value is obtained.

It can be seen in Fig. 2 that the lactate formed arises from both the pyruvate formed by glycolysis and the pyruvate synthesized from oxaloacetate by the action of phosphoenolpyruvate carboxykinase and pyruvate kinase. Let us call $X_p$ the net amount of C-2 of lactate synthesized by lactate dehydrogenase from the pyruvate derived from glycolysis. Therefore,

$$[C_2\text{Lac}]^{C_2\text{Glc}} = X_p l + [C_2\text{OAA}]^{C_2\text{Glc}} b r l.$$  

Thus

$$X_p l = [C_2\text{Lac}]^{C_2\text{Glc}} - [C_2\text{OAA}]^{C_2\text{Glc}} b r l  \quad \text{(Eq. 2)}$$

where

$$[C_2\text{OAA}]^{C_2\text{Glc}} b r l = \frac{[C_2\text{Lac}]^{C_2\text{Glc}}}{[C_2\text{OAA}]^{C_2\text{Glc}}} \cdot [C_2\text{Glc}]^{C_2\text{Glc}}  \quad \text{(Eq. 3)}$$

Fig. 2 also shows that the ratio

$$\frac{[C_2\text{Gluc3P} + \text{Gro}]}{[C_2\text{Lac}]^{C_2\text{Glc}}} = j v' r l  \quad \text{(Eq. 4)}$$

where $j = (1 - r)$, because phosphoenolpyruvate is either converted into pyruvate (proportion $r$) or enters the gluconeogenic pathway (proportion $j$) and $v'$ is the proportion of triose phosphate reconverted into glycerol 3-phosphate plus glycerol after having passed through the stage of oxaloacetate.

The amount of the phosphoenolpyruvate-derived glycerol 3-phosphate and glycerol labeled on their C-2 ($\text{C}_2\text{OAA}$ and $\text{C}_2\text{Gro}$) when [2-13C]glucose is the substrate (see Fig. 2) can be calculated from Equations 3 and 4 and is shown in Equation 5.

$$[C_2\text{OAA}]^{C_2\text{Glc}} b r l v' = [C_2\text{Lac}]^{C_2\text{Glc}} b r l j v' r l  \quad \text{(Eq. 5)}$$

As indicated in Table I, $w$ and $v$ are the proportions of triose phosphate formed only by glycolysis and reconverted into glucose and into glycerol 3-phosphate plus glycerol, respectively; the proportions of triose phosphate reconverted into glucose and into glycerol 3-phosphate plus glycerol after having passed through the stage of oxaloacetate are defined as $w'$ and $v'$, respectively.

It can be seen from Fig. 2 that:

$$w v' = w' v = 2[C_2\text{Glc}]^{C_2\text{Glc}}(C_2\text{Gro3P} + \text{Gro})^{C_2\text{Glc}}  \quad \text{(Eq. 6)}$$

Since $v' + w' = 1$, $w' = 1/\left[1 + (w'/w')\right]$; therefore, the value of $w'$ can be obtained. In order to calculate $v$ and $w$, one needs to determine the amount of glucose plus glycerol 3-phosphate and glycerol synthesized from the triose phosphate formed only by glycolysis. From Fig. 2, it can be seen that:

$$X_p (w + v) = 2\left([C_2\text{Glc}]^{C_2\text{Glc}} + [C_2\text{Gro3P} + \text{Gro}]^{C_2\text{Glc}}\right) - [C_2\text{OAA}]^{C_2\text{Glc}} b r l j$$

$$\quad \text{(Eq. 7)}$$

Since $X_p (w + v)$ can be obtained in the same manner as $w v'$ is also known (see above), $v = (w + v')/\left[1 + (w'/v')\right]$ and the value of $w$ can be obtained.

This allows calculation of $p$, which is the proportion of triose phosphate converted into pyruvate by glycolysis because $w + v + p = 1$ (see Fig. 2).

Given the values of $X_p l$ (Eq. 2) and $X$ and $p$ (see above) are known, one can deduce the value of $l$, which is the proportion of pyruvate converted into lactate.

In order to determine $r$, the ratio $j r l$ should be calculated.

$$j r l = \frac{[C_2\text{Lac}]^{C_2\text{Glc}} + [C_2\text{OAA}]^{C_2\text{Glc}} + [C_2\text{Gro3P} + \text{Gro}]^{C_2\text{Glc}}}{[C_2\text{Lac}]^{C_2\text{Glc}}}  \quad \text{(Eq. 8)}$$

![Figure 4](https://example.com/fig4.png)

**Fig. 4. Schematic representation of the multicycle operating during glucose metabolism in rabbit kidney tubules. The figure represents the three interdependent cycles whose common metabolite is oxaloacetate. These cycles are: (i) the tricarboxylic acid cycle, in which a proportion $g$ of oxaloacetate is recycled at each turn, (ii) the "OAA → PEP → Pyr → OAA" cycle, in which a proportion $h$ of oxaloacetate is recycled at each turn, and (iii) the "OAA → PEP → Pyr → AcCoA → citrate → OAA" cycle, in which a proportion $z$ of oxaloacetate is recycled at each turn. The proportion $e$ takes into account the glucose-derived acetyl-CoA molecules that condense with endogenous oxaloacetate molecules to give citrate. This figure, like Fig. 2, shows the phosphoenolpyruvate formed by the phosphoenolpyruvate carboxykinase reaction but not that formed by glycolysis.**

**Table I**

| Parameter | Definition of the parameters of the model | Converted to | Parameter notation |
|-----------|------------------------------------------|--------------|------------------|
| OAA       | Citrate                                  | $a$          |                  |
| OAA       | PEP                                      | $b$          |                  |
| Pyr       | OAA                                      | $c$          |                  |
| Pyr       | AcCoA                                    | $d$          |                  |
| PEP       | Pyr                                      | $e$          |                  |
| PEP       | Triglycer-P                               | $j$          |                  |
| OAA-derived trioses-P | Gro3P + Gro | $k$          |                  |
| OAA-derived trioses-P | Glc | $l$          |                  |
| OAA-derived trioses-P | OAA | $m$          |                  |
| Other parameters |                      |              |                  |
| Glu         | Glu accumulated                           | $q$          |                  |
| Glu         | Glu accumulated                           | $t$          |                  |

Since $l$ is known, one can deduce $j r l$ and since $j = (1 - r)$ (see Fig. 2),

$$r = 1/[j r l + 1]  \quad \text{(Eq. 9)}$$

Let us call $h$ the proportion of the oxaloacetate that is synthesized (or recycled) at the end of each turn of the cycle "OAA → PEP → Pyr → OAA" (shown in Fig. 4); it can be seen in Fig.
Fig. 5. Schematic representations of oxaloacetate formation during the first and second multicycle turns. This figure contains another representation of Fig. 4 (left panel) which allows, as shown for the first and second multicycle turns (right panel), to calculate the total amount of oxaloacetate formed during an infinite number of multicycle turns.

4 and Table I that \( h = b \cdot r \cdot c \). Several steps of calculations are required to obtain the value of \( h \) (see Fig. 2).

\[
[C_3OAA]^{C12}\text{C2G}\text{C} = X_p c \cdot (1 - i - h) / [1 - (1 - i - h)] \tag{Eq. 10}
\]

Since \( h = b \cdot r \cdot c \) it follows that:

\[
[C_3Lac]^{C12}\text{C2G}\text{C} = X_p l + [C_3OAA]^{C12}\text{C2G}\text{C} \tag{Eq. 11}
\]

Since \( X_p l \) is known and \( i \) is given an arbitrary value then, \( h \) can be calculated.

It should be stressed that the final value of \( h \) is obtained by iterations, using Equations 23 and 24.

The proportion of pyruvate converted into phosphoenolpyruvate, equal to \( c \cdot b \), can be calculated by dividing \( h \) by \( r \) because \( h = c \cdot b \cdot r \).

The proportions of pyruvate converted into citrate and into acetyl-CoA, equal to \( c \cdot a \) and \( d \), respectively, can be calculated thanks to the two equations that follow (see Fig. 2).

\[
d + c \cdot a = (1 - i) - c \cdot b \tag{Eq. 13}
\]

\[
dc \cdot a = [C_3Glx]^{C12}\text{C2G}\text{C} / [C_3Glx]^{C12}\text{C2G}\text{C} + [C_3Glx]^{C12}\text{C2G}\text{C} \tag{Eq. 14}
\]

Then,

\[
c \cdot a = (d + c \cdot a) / [1 + (dc \cdot a)] \tag{Eq. 15}
\]

Given that \( c \cdot a \) is known, one can deduce the value of \( d \).

The knowledge of \( c \cdot b \) and of \( c \cdot a \) (see above) allows calculation of the value of \( c \), the proportion of the pyruvate converted into oxaloacetate by pyruvate carboxylase, because \( c = c \cdot a + c \cdot b \) and \( a + b = 1 \) (see Fig. 2). Then, since \( c, c \cdot a, c \cdot b \) are known, \( a \) and \( b \), the proportions of oxaloacetate converted into citrate and phosphoenolpyruvate, respectively, can be calculated.

The proportion of \( \alpha \)-ketoglutarate converted into oxaloacetate, which is equal to \( s \), can be calculated thanks to the labeling of the C-5 of Glx and of the C-2 of lactate from [2-13C]glucose by using the following equation.

\[
d(1 - s) = [C_3Glx]^{C12}\text{C2G}\text{C} / [C_3Lac]^{C12}\text{C2G}\text{C} \tag{Eq. 16}
\]

Since \( i \) and \( d \) are known, \( s \) can be calculated.

From \( a \) and \( s \), it is possible to calculate \( g \), which is the proportion of oxaloacetate recycled at each tricarboxylic acid cycle turn (see Fig. 4 and Table I), because \( g = a \cdot s \).

From \( b, r, d, \) and \( s \), it is possible to calculate \( z \), which is the proportion of oxaloacetate recycled at each “Pyr → AcCoA → citrate → OAA → PEP → Pyr” cycle turn (see Fig. 4 and Table I), because \( z = b \cdot r \cdot d \cdot s \).

The proportions \( q \) and \( t \) of the \( \alpha \)-ketoglutarate leaving the tricarboxylic cycle to accumulate as glutamate and glutamine, respectively, are obtained as follows.

\[
q = [C_3Glx]^{C12}\text{C2G}\text{C} / [C_3Glx]^{C12}\text{C2G}\text{C} \tag{Eq. 17}
\]

\[
t = 1 - q \tag{Eq. 18}
\]

The determination of \( i \) is possible by using the pieces of information contained in Figs. 2 and 3 concerning the labeling of the C-2 and C-3 of Glx which are derived from the labeling of the C-3 and C-2 of oxaloacetate, respectively. From Fig. 3, which shows the C-1, C-2, C-3, and C-4 of oxaloacetate formed during the two first turns of the multicycle represented in Fig. 4, one can determine the amount of labeled C-2 of oxaloacetate formed from glucose labeled on its C-2 and C-1 by extrapolating to an infinite number of turns.

This leads to the following equations.

\[
[C_3OAA]^{C12}\text{C2G}\text{C} = X_p c \cdot [(1 - i) + i \cdot [K]] / [Q] \tag{Eq. 19}
\]

where

\[
[K] = [g/2 + i \cdot h] / ([g/2 + (1 - i) \cdot h]) \tag{Eq. 20}
\]

and

\[
[Q] = 1 - [[g/2 + (1 - i) \cdot h] + [g/2 + i \cdot h] / (1 - [g/2 + (1 - i) \cdot h])] \tag{Eq. 21}
\]

Since \( [C_3Glx]^{C12}\text{C2G}\text{C} / [C_3Glx]^{C12}\text{C2G}\text{C} \) is equal to \( [C_3OAA]^{C12}\text{C2G}\text{C} / [C_3OAA]^{C12}\text{C2G}\text{C} \), one can deduce that

\[
[C_3Glx]^{C12}\text{C2G}\text{C} / [C_3Glx]^{C12}\text{C2G}\text{C} = ([i + (dc \cdot (s/2))] + [(1 - i) + (dc \cdot (s/2))] \cdot [K] / (1 - (1 - i) \cdot [K]) \tag{Eq. 23}
\]

The same kind of reasoning allows one to obtain Equation 24,

\[
[C_3Glx]^{C12}\text{C2G}\text{C} / [C_3Glx]^{C12}\text{C2G}\text{C} = ([1 - i + (dc \cdot (s/2))] + [i + (dc \cdot (s/2))] \cdot [K] / (1 - (1 - i) \cdot [K]) \tag{Eq. 24}
\]

where \( [K'] = [g/2 + i \cdot h] / ([g/2 + (1 - i) \cdot h]). [C_3Glx]^{C12}\text{C2G}\text{C} / [C_3Glx]^{C12}\text{C2G}\text{C} \) and \( [C_3Glx]^{C12}\text{C2G}\text{C} / [C_3Glx]^{C12}\text{C2G}\text{C} \) ratios calculated from an arbitrary value of \( i \) are compared with their corresponding experimental ratios, allowing one to obtain the final value of \( i \) after several successive adjustments.

Calculations of the Enzymatic Fluxes—In our model, as already mentioned, a flux through a given enzyme is taken as the formation per unit of time of one product of the reaction catalyzed by this enzyme. It should be pointed out that oxaloacetate is the only metabolite common to the first three metabolic cycles involved in glucose metabolism. Therefore, a key step in the calculations of enzymatic fluxes is the determination of the amount of the oxaloacetate molecules that have been formed in relation to glucose metabolism (noted [OAA]G12). These oxaloacetate molecules containing 1, 2, 3, 4, or 0 carbon atoms derived from glucose. Fig. 5 gives a schematic representation providing the basic elements needed for such a determination. In the left panel of Fig. 5, which is derived from Fig. 4, \( h, g, \) and \( z \) represent the oxaloacetate recycled in the “OAA → PEP → Pyr → OAA” cycle, in the tricarboxylic acid cycle, and in the “Pyr → AcCoA → citrate → OAA → PEP → Pyr” cycle, respectively (see also Fig. 4). Let us call \( e \) the proportion of the acetyl-CoA molecules derived from glucose that have been condensed with oxaloacetate molecules of endogenous origin to give citrate. It is necessary to introduce this proportion (see Fig. 4) to calculate correctly the oxaloacetate formation from glucose by avoiding to take into
account twice the citrate molecules synthesized from an oxaloacetate and an acetyl-CoA molecules originating both from glucose. The proportion e also allows one to take into account the oxaloacetate molecules formed from an acetyl-CoA molecule derived from glucose and an oxaloacetate molecule arising from endogenous substrates. Thus, the proportion of acetyl-CoA derived from glucose and condensed with endogenous oxaloacetate to give oxaloacetate via the tricarboxylic acid cycle is equal to e (Figs. 4 and 5). Then, at each turn of the multicycle, the additional proportion of oxaloacetate formed as a result of the operation of the “Pyr → AcCoA → citrate → OAA → PEP → Pyr” cycle is e \( \sigma \), while the proportion of oxaloacetate formed by the “OAA → PEP → Pyr → OAA” cycle and by the tricarboxylic acid cycle are \( h \) and \( g \), respectively.

The right panel of Fig. 5 summarizes the oxaloacetate formation from glucose shown in more detail in the left panel of the same figure and allows to calculate the total amount of oxaloacetate derived from glucose ([OAA]G) using the following equations derived from Fig. 5, in which the repetitiveness of the formation of oxaloacetate by the operation of the three cycles is taken into account thanks to the parameter \( h + g + e \), which represents the proportion of oxaloacetate recycled at each turn of the multicycle presented in Fig. 4.

\[
[\text{OAA}]_{G_e} = 2X_p(c + d + e)v \sum (h + g + e)v^x
\]

(Eq. 25)

\[
[\text{OAA}]_{G_e} = 2X_p(c + d + e)v / (1 - h - g - e)v
\]

(Eq. 26)

In these equations, the parameter \( 2X \) is used to take into account the fact that during glycolysis, two molecules of triose phosphate are formed from one glucose molecule.

In the latter equation, \( I(1 - h - g - e)v \) represents the proportion of the oxaloacetate recycled for an infinite number of multicycle turns, i.e. the oxaloacetate turnover. Using the NMR data obtained with \([1-\text{13C}]\text{glucose} \) as substrate, the value of \( e \) can be obtained by calculating first \( 1 - e \), which represents the proportion of acetyl-CoA molecules derived from glucose that has been condensed with oxaloacetate molecules also derived from glucose. This proportion can be assessed by the ratio of \([2-\text{13C}]\text{acetyl-CoA} \), which condenses with \([2-\text{13C}]\text{oxaloacetate} \) and oxaloacetate (a ratio reflected by the proportion of the C-4 and C-3 of Glx found to be coupled on the NMR spectra and corrected to take into account the total oxaloacetate formation from glucose). Thus, \( (1 - e) = ([C_4\text{Glx}]_{[\text{C_4Glx}]} + [C_2\text{Glx}]_{[\text{C_2Glx}]}) / [\text{OAA}]_{[\text{OAA}]^C = (1 - s) / [C_4\text{Glx}]_{[\text{C_4Glx}]} \) Since, in our experimental conditions, the C-1 and C-6 of glucose have virtually the same fates, it follows that: \( (1 - e) = ([C_4\text{Glx}]_{[\text{C_4Glx}]} + [C_2\text{Glx}]_{[\text{C_2Glx}]}) / ([\text{OAA}]_{[\text{OAA}]^C = (1 - s) / [C_4\text{Glx}]_{[\text{C_4Glx}]}) \), where \([\text{OAA}]_{[\text{OAA}]^C = (1 - s) = [\text{Glx}]_{[\text{Glx}]^C} \)

In view of the fact that \([\text{OAA}]_{[\text{OAA}]^C \mathbb{E} and e \) are not independent parameters, they should be calculated by iterations.

Since the activity of malic enzyme is considered to be negligible in rabbit kidney tubules (1), one can write that: \([\text{OAA}]_{[\text{OAA}]^C = \text{flux through pyruvate carboxylase + flux through a-keto-glutarate dehydrogenase.} \)

Flux through pyruvate dehydrogenase, which is equal to \([\text{AcCoA}]_{[\text{AcCoA}]^C \mathbb{E} \text{can be derived from Fig. 4: } [\text{AcCoA}]_{[\text{AcCoA}]^C = 2X_p(d + [\text{OAA}]_{[\text{OAA}]^C,b} \mathbb{E} \text{Flux through phosphoenolpyruvate carboxylase is given by } [\text{OAA}]_{[\text{OAA}]^C,b} \mathbb{E} \text{Flux through pyruvate kinase is equal to } 2X_p + [\text{OAA}]_{[\text{OAA}]^C,b} \mathbb{E} \text{Flux through lactate dehydrogenase, obtained by multiplying flux through pyruvate kinase by } l \), is equal to \( (2X_p + l + [\text{OAA}]_{[\text{OAA}]^C,b} \mathbb{E} \text{Flux through glycerol-3-phosphate dehydrogenase is given by the following formula: } (2X_p + [\text{OAA}]_{[\text{OAA}]^C,b} \mathbb{E}) \]

Flux through glucose-6-phosphatase is equal to \( 2X_p + [\text{OAA}]_{[\text{OAA}]^C,b} \mathbb{E} \text{and corresponds to } 2(\text{Glc})_{\mathbb{E} \text{since fluxes are expressed in C3 units.} \)

Flux through citrate synthase is given by: \([\text{citrate}]_{[\text{citrate}]^C = [\text{OAA}]_{[\text{OAA}]^C,a} \mathbb{E} + [\text{AcCoA}]_{[\text{AcCoA}]^C} \mathbb{E} \) (see Fig. 4). Note that this equation takes into account the condensation of endogenous oxaloacetate molecules with labeled glucose-derived acetyl-CoA molecules.

Flux through glutamate dehydrogenase is equal to the flux through citrate synthase multiplied by \((1 - s) \) (see Fig. 2).

Flux through glutamine synthease is equal to the flux through glutamate dehydrogenase multiplied by \( t \) (see Fig. 2).

Flux through \( \alpha \)-ketoglutarate dehydrogenase, which is taken as flux through the Krebs cycle, is obtained by multiplying by \( s \) the flux through citrate synthase (see Figs. 2 and 4).

Flux through pyruvate carboxylase is equal to \([\text{OAA}]_{[\text{OAA}]^C \mathbb{E} \text{minus flux through } \alpha \text{-ketoglutarate dehydrogenase.} \)

Finally, since the amount of glucose resynthesized by gluconeogenesis and further metabolized was necessarily very small (because the rate of gluconeogenesis was small) and taking into account that all fluxes are expressed in C3 units, flux through hexokinase can be taken as \( 2X \) (see Fig. 2 and Equation 1).

It is easy to demonstrate that the inversion, \( i \), between the C-2 and the C-3 of oxaloacetate observed after the equilibration with fumarate, which results from a great number of passages through the stage of fumarate, is the same as that occurring at each passage through the stage of fumarate.

Flux of equilibration of oxaloacetate with fumarate during the first passage is equal to the oxaloacetate derived from glucose multiplied by twice the inversion, that is \([\text{OAA}]_{[\text{OAA}]^C = 2i} \mathbb{E} \) At each subsequent passage, a proportion \( 2i \) of the flux of equilibration during the preceding passage undergoes again equilibration. Therefore, flux of equilibration of oxaloacetate with fumarate after an infinite number of passages is equal to \([\text{OAA}]_{[\text{OAA}]^C = 2i} \mathbb{E} \) multiplied by \( 1/(1 - 2i) \)

Thus, flux of oxaloacetate equilibration with fumarate is equal to \([\text{OAA}]_{[\text{OAA}]^C = 2i} \mathbb{E} / (1 - 2i) \)

DISCUSSION

Our model, which can be used under non-steady state conditions, is based on proportions of metabolite conversion. Therefore, it cannot be directly compared to other models (2–11), which are based on kinetic reaction rates and require steady state conditions.

Most of the proportions and equations we used to calculate enzymatic fluxes were derived from the fate of the C-2 of glucose, which provides more information about all Krebs cycle turns than that of the C-3 of glucose, which is released as CO2 and recovered in the non-volatile products of glucose metabolism that accumulate during the first Krebs cycle turn. In the present study, the data obtained with \([1-\text{13C}]\text{glucose} \) as substrate were used only in combination with those obtained when \([2-\text{13C}]\text{glucose} \) was the substrate to calculate the equilibration of oxaloacetate with fumarate. It should be emphasized that many proportions could also have been calculated from the data obtained with \([1-\text{13C}]\text{glucose} \) as substrate by using equations similar to those presented in the model of \([2-\text{13C}]\text{glucose} \) metabolism. This illustrates the flexibility of our mathematical model, which can also be applied to data obtained with substrates other than glucose and under many physiological conditions. Finally, depending on the experimental data available, it allows to calculate either net or unidirectional enzymatic fluxes.

REFERENCES

1. Waford, M., Vinay, P., Lernieux, G., and Gogoua, A. (1980) Biochem. J. 188, 741–748
Model of Glycolysis, Gluconeogenesis, and Citric Acid Cycle

2. Magnusson, I., Schumann, W. C., Bartsh, G. E., Chandramouli, V., Kumaran, K., Wahren, J., and Landau, B. R. (1991) J. Biol. Chem. 266, 6975–6984
3. Cohen, S. M. (1987) Biochemistry 26, 581–589
4. Malloy, C. R., Sherry, A. D., and Jeffrey, F. M. H. (1990) J. Biol. Chem. 265, 6964–6971
5. Malloy, C. R., Sherry, A. D., and Jeffrey, M. H. (1990) Am. J. Physiol. 259, H987–H996
6. Katz, J., Lee W. N. P., Wals, P. A., and Bergmeyer, H. (1989) J. Biol. Chem. 264, 12924–12931
7. Donato, L., Des Rosiers, C., Montgomery, J. A., David, F., Garneau, M., and Brunengraber, H. (1993) J. Biol. Chem. 268, 4170–4180
8. Rognstad, R., and Katz, J. (1972) J. Biol. Chem. 247, 6047–6054
9. Kelleher, J. R. (1985) Am. J. Physiol. 248, E252–E260
10. Mason, G. F., Rothman, D. L., Behar, K. L., and Shulman, R. G. (1992) J. Cereb. Blood Flow Metab. 12, 434–447
11. Chance, E. M., Stohlker, S. H., Kobayashi, K., and Williams, J. R. (1983) J. Biol. Chem. 258, 13785–13794
12. Chauvin, M.-F., Mégmin-Chanet, F., Martin, G., Lhoste, J-M., and Baverel, G. (1994) J. Biol. Chem. 269, 26925–26933