Model Applicable to NMR Studies for Calculating Flux Rates in Five Cycles Involved in Glutamate Metabolism

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Guy Martin‡, Marie-France Chauvin, and Gabriel Baverel

From the Centre d’Études Métaboliques par Spectroscopie de Résonance Magnétique (INSERM CRI 950102), Hôpital Édouard Herriot, 69374 Lyon Cedex 03, France

Based on the same principles as those utilized in a recent study for modeling glucose metabolism (Martin, G., Chauvin, M. F., Dugelay, S., and Baverel, G. (1994) J. Biol. Chem. 269, 26034–26039), a method is presented for determining metabolic fluxes involved in glutamate metabolism in mammalian cells. This model consists of five different cycles that operate simultaneously. It includes not only the tricarboxylic acid cycle, the “oxaloacetate → phosphoenolpyruvate → pyruvate → oxaloacetate” cycle and the “oxaloacetate → phosphoenolpyruvate → pyruvate → acetyl-CoA → citrate → oxaloacetate” cycle but also the “glutamate → α-ketoglutarate → glutamate” and the “glutamate → glutamine → glutamate” cycles. The fates of each carbon of glutamate, expressed as ratios of integrated transfer of this carbon to corresponding carbons in subsequent metabolites, are described by a set of equations. Since the data introduced in the model are micrograms of atom of traced carbon incorporated into each carbon of end products, the calculation strategy was determined on the basis of the most reliable parameters determined experimentally. This model, whose calculation routes offer a large degree of flexibility, is applicable to data obtained by 13C NMR spectroscopy, gas chromatography–mass spectrometry, or 14C counting in a great variety of mammalian cells.

In the accompanying paper (16), we have conducted a study on glutamate 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 the calculation of reaction rates of gluconeogenesis, tricarboxylic acid cycle, and the pathways of glutamate and glutamine synthesis and degradation occurring simultaneously in mammalian cells. This model, which is applicable to data obtained by 13C NMR, gas chromatography–mass spectrometry, and 14C counting, is described in the present paper.

THEORY

Schematic Representation of Glutamate Metabolism—A general representation of glutamate metabolism is given in Fig. 1. This figure shows the main pathways of glutamate metabolism, as well as the main products accumulated during glutamate metabolism.

Fig. 2 shows five metabolic cycles that are functioning simultaneously during glutamate metabolism. Oxaloacetate is the only metabolite common to three of these cycles that were referred to as a multicycle in a previous study (1) and are (i) the tricarboxylic acid cycle, (ii) the “OAA1 → PEP1 → Pyr1 → OAA” cycle and (iii) the “OAA → PEP → Pyr → AcCoA1 → Cit1 → OAA” cycle.

Glutamate is the metabolite common to the two other cycles that have been introduced to improve the model; these are (iv) the “Glu → αKG → Glu” cycle and (v) the “Glu → Gln → Glu” cycle.

Fig. 3, derived from Fig. 2, allows the calculation of the total amount of oxaloacetate formed from glutamate during 1 h of incubation and, subsequently, the calculation of the amount of the different intermediates and end products formed from glutamate during the same incubation time. From these data, fluxes can be calculated since a flux through a given enzyme is taken as the formation of one product of the reaction catalyzed by this enzyme during 1 h of incubation.

In our model, the calculation of the proportions of each metabolite converted into the next one(s) is based on the fates of individual carbons 3, 5, and 1 of the glutamate molecule together with the fate of the incorporated labeled CO2 which are represented in Figs. 4 and 5, respectively.

Fig. 6 shows the successive proportions allowing us to calculate the amount of labeled oxaloacetate formed from labeled glutamate. These proportions are related to the substrates and not to the products of the reactions.

Notations—Let us call [C13C15O2]Glu the amount of the metabolite (MET) labeled on its carbon y (where 1 ≤ y ≤ 6) arising from glutamate labeled on its carbon z, where z is equal to 1, 3, or 5 because we used [1,2-13C]-, [1-14C]-, [3-13C]-, [5-13C]-, [1,5-14C]-, and [U-14C]glutamate as labeled substrates and also because it is assumed that the C-2 and C-3 of glutamate had the same metabolic fate as the C-5 and C-4, respectively.

And let [C15O2]Glu+CO2 be the amount of the metabolite (MET) labeled on its carbon y (where 1 ≤ y ≤ 6) arising from glutamate plus labeled CO2.

Similarly, let [(C15O2)+C13C15O2]MET be the amount of the metabolite (MET) labeled on its carbon y plus the amount of the metabolite (MET) labeled on its carbon y′; let [(C15O2)+C15O2]MET be the amount of the metabolite (MET) labeled simultaneously on its carbons y and y′.

Let [C15O2](MET1 + MET2) be the amount of the metabo-

1 The abbreviations used are: OAA, oxaloacetate; Ac, acetate; AcCoA, acetyl-coenzyme A; Asp, aspartate; Cit, citrate; CS, citrate synthase; Glc, glucose; Glu, glutamine; Glnase, glutaminase; Glu, glutamate; Glx, glutamate + glutamine; GS, glutamine synthetase; αKG, α-ketoglutarate; αKGdH, α-ketoglutarate dehydrogenase; Lac, lactate; PEP, 3-phosphoglycerate; PEP, phosphoenolpyruvate; Pyr, pyruvate; TCA, tricarboxylic acid, MET, metabolite; Ser, serine.

‡ To whom correspondence should be addressed: Centre d’Études Métaboliques par Spectroscopie de Résonance Magnétique, Pavillon P, Hôpital Édouard Herriot, place d’Arsonval, 69374 Lyon Cedex 03, France. Tel.: (33) 04-78-77-86-65; Fax: (33) 04-78-77-87-39.

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1 The abbreviations used are: OAA, oxaloacetate; Ac, acetate; AcCoA, acetyl-coenzyme A; Asp, aspartate; Cit, citrate; CS, citrate synthase; Glc, glucose; Glu, glutamine; Glnase, glutaminase; Glu, glutamate; Glx, glutamate + glutamine; GS, glutamine synthetase; αKG, α-ketoglutarate; αKGdH, α-ketoglutarate dehydrogenase; Lac, lactate; PEP, 3-phosphoglycerate; PEP, phosphoenolpyruvate; Pyr, pyruvate; TCA, tricarboxylic acid, MET, metabolite; Ser, serine.
Model of Glutamate Metabolism

Fig. 1. Pathways of glutamate metabolism in rabbit kidney tubules. Glutamine which enters the cell can be accumulated or converted by glutamine synthetase into glutamine which can accumulate or be reconverted into glutamate by glutaminase. Glutamate can also be converted into α-ketoglutarate either by glutamate dehydrogenase or alanine aminotransferase or aspartate aminotransferase or phosphoserine aminotransferase. The α-ketoglutarate formed is either reconverted into glutamate mainly by glutamate dehydrogenase or enters the tricarboxylic acid cycle to give oxaloacetate after having lost one carbon as CO₂. The oxaloacetate formed after transamination with glutamate by aspartate aminotransferase yields aspartate. Oxaloacetate can also be converted into phosphoenolpyruvate, thanks to the phosphoenolpyruvate carboxykinase reaction, or condense with acetyl-CoA to give citrate and, after decarboxylation, regenerate α-ketoglutarate and therefore complete one tricarboxylic acid cycle turn. In the presence of NH₄⁺, part of this α-ketoglutarate may be reconverted into glutamate resulting, as already mentioned, in accumulation of glutamate or glutamine. The phosphoenolpyruvate formed may be converted into pyruvate by pyruvate kinase, or into glucose by the gluconeogenic pathway, or into serine. Pyruvate, after transamination with glutamate by alanine aminotransferase, yields alanine. Pyruvate can also be accumulated as lactate by lactate dehydrogenase or, after decarboxylation by pyruvate decarboxylase, converted into acetyl-CoA. This acetyl-CoA together with acetyl-CoA originating from endogenous sources and from exogenous acetate, when added to the incubation medium, is condensed to oxaloacetate to give citrate. The amount per g dry wt of added glutamate and added acetate utilized during 1 h of incubation are designed by X and Y, respectively. The notations of the proportion of a metabolite directly converted into the subsequent one(s) is simply given by the figure and can be represented by (precursor metabolite → derived metabolite). The notations taking into account the recycling over an infinite number of turns of exclusively the “Glu → αKG → Glu” and “Glu → Gln → Glu” cycles is indicated by substituting the parentheses ( ) by [ ]

The corresponding proportion taking into account the recycling over an infinite number of turns of exclusively the “Glu → αKG → Glu” and “Glu → Gln → Glu” cycles is indicated by substituting the parentheses ( ) by [ ].

Special symbols are used in the notations [“Glu → Met2] and [c₆OAcαKG → OAA] to stress the fact that only added glutamate and only citrate-derived α-ketoglutarate, respectively, are concerned.

Let (AcCoA + OAA → Cit) be the proportion of acetyl-CoA related to glutamate metabolism that is condensed to oxaloacetate not related to glutamate metabolism to yield direct synthesis of citrate at each turn of the TCA cycle. Similarly, let (OAA + AcCoA → Cit) the proportion of oxaloacetate related to glutamate metabolism which is condensed to acetyl-CoA not related to glutamate metabolism to yield direct synthesis of citrate at each turn of the TCA cycle.

Let [Met1 → Met2] be the flux of conversion of Met1 to Met2. The model of glutamate metabolism which include five main metabolic cycles as presented in Fig. 2.

Calculations of the Parameters of the Model—The different notations employed in the figures and in the text to characterize the parameters of our model are also defined in Table I.

(RR) = ∑(RF)ₙ = 1/[1 - (RF)]

A complete turn of the metabolic cycle is considered to have occurred as soon as the metabolite of interest of the cycle is resynthesized once.
The parameters \([\text{Glu} \rightarrow \text{Gln}]\) and \([\text{Glu} \rightarrow \text{Gln}]\) as defined above (see “Notations”), are equal to \([\text{Glu} \rightarrow \text{Gln}]\) multiplied by \((\text{Glu} \rightarrow \text{Gln})\) and \((\text{Glu} \rightarrow \text{Gln})\), respectively.
Fig. 4. Metabolic fate of the C-5, C-3, and C-1 of glutamate in rabbit kidney tubules. This figure shows the metabolic fate of glutamate labeled either on its carbon 5, 3, or 1 which, for sake of simplicity, is represented as 5,3,1 GLU. Glutamate metabolites are represented as α, β, γ MET, where MET represents any glutamate-derived metabolite and α, β, and γ the labeled carbon of these metabolites when the labeled carbon of the glutamate added as substrate was 5, 3, or 1, respectively. Unlabeled carbons of glutamate metabolites are represented by a minus sign. The amount (in μmol dry wt/h) of labeled glutamate utilized is represented by X. The proportion of the direct conversion of a metabolite to the next one is indicated by a simple arrow with no special mention. To take into account the fact that some reactions yield a metabolite labeled at two different positions, it is necessary to multiply the proportion of conversion by the proper factor 1/2 or (OAA) or 1 - (OAA), as indicated in the figure. For other metabolic conversions it is necessary to take into account the effect of the recycling through the “glutamate” cycles. Depending on which metabolite is recycled, glutamate or α-ketoglutarate, the proportion of conversion is multiplied either by [Glu %] αKG + Gln] or [αKG %] Gln + Gln] (see Table I) which are represented by specific arrows consisting of a double line and a dash-stacked line, respectively. For sake of clarity, only direct formation of α-ketoglutarate from citrate is shown in this figure. To take into account the recycling of α-ketoglutarate through glutamate and glutamine, it is necessary to multiply by [αKG %] Glu + Gln].

The proportion of oxaloacetate resynthesized at each turn of this cycle (see Fig. 2) is equal to (OAAi), as indicated in the figure. To take into account the recycling of α-ketoglutarate through glutamate and glutamine, it is necessary to multiply by [αKG %] Glu + Gln].

The amount of glutamine formed directly from the substrate glutamate is given by X(3Glu → Gln), where X is the amount of glutamate utilized and [3Glu → Gln] is the proportion of the substrate glutamate converted into glutamine. As already mentioned, this proportion takes into account glutamate recycling through α-ketoglutarate and glutamine.

The corresponding amount of glutamine accumulated is given by X(3Glu → Gln) = X(Glu → 3Gln)(Glu % αKG + Gln) where (Glu → 3Gln) = (Glu → Gln) + (Gln → 3Gln) and (Gln → 3Gln) is the proportion of glutamine formed which accumulated (see also Equation 3).

It can be seen in Fig. 4 that X(3Glu → Gln) is given by C3Gln accumulated from C3Glu: X(3Glu → Gln) = [C3Gln]C3Glu -...
4. Oxaloacetate carbons directly labeled from the CO₂ carbon are represented in Fig. 4. We can calculate which is fixed by pyruvate carboxylase at each turn of the Pyr of multicycle turns. Then the notation of different proportions are explained in the figure, derived from Fig. 4, allowing the calculation of the amounts of the \( [\text{C}_2\text{Glu}] \) and \( [\text{C}_3\text{Glu}] \) where (OAA) is the proportion of oxaloacetate inverted as a glutamate metabolite, \( X \) is equal to \( (\text{Glu} \rightarrow \alpha\text{KG}) \) and (OAA) is the proportion of oxaloacetate inverted as a glutamate metabolite, \( X \) is equal to (Glu \rightarrow \alpha\text{KG})/(Glu + \alpha\text{KG} + \text{Gln})

\[ [\text{C}_2\text{Glu}]^{\text{Glu}} / [\text{C}_2\text{Glu}]^{\text{Gln}} \] and \( [\text{C}_3\text{Glu}]^{\text{Glu}} / [\text{C}_3\text{Glu}]^{\text{Gln}} \) is equal to the ratio \( \text{Glu} / \text{Gln} \) (see above, Figs. 2 and 4 and Table I). From \( X \) and \( \alpha\text{KG} \), one can calculate \( X \) and \( \alpha\text{KG} \).

Fig. 2 shows that the glutamate utilized (X) is either accumulated as glutamate, \( X \) is equal to \( \alpha\text{KG} \), or glutamine, \( X \) is equal to \( \alpha\text{KG} \), or converted into oxaloacetate, \( X \) is equal to \( \alpha\text{KG} \) (see also Table I), and where as mentioned above (Pyr \ text{PEP}) = (OAA \ text{PEP}) \ (Pyr \ text{PEP}) \ (Pyr \ text{OAA}) \ (see also Fig. 2 and Table I).

\[ [\text{C}_3\text{OAA}]^{\text{Glu}} = X \cdot \alpha\text{KG} \cdot (\alpha\text{KG} \rightarrow \text{OAA}) / 2 \]

\[ + (\text{OAA}) \cdot (\text{Pyr} \ | \text{OAA}) \cdot [\text{C}_3\text{OAA}]^{\text{Glu}} = (1 - [\text{OAA}]) \cdot (\text{Pyr} \ | \text{OAA}) \cdot [\text{C}_3\text{OAA}]^{\text{Glu}} \]

Therefore the following parameters can be calculated:

\[ (\text{Glu} \rightarrow \alpha\text{KG}) \cdot (\alpha\text{KG} \rightarrow \text{OAA}) \]

\[ = X \cdot \alpha\text{KG} \cdot (\alpha\text{KG} \rightarrow \text{OAA}) \]

\[ = X \cdot \alpha\text{KG} \cdot (\alpha\text{KG} \rightarrow \text{OAA}) \]

where (OAA) is the proportion of oxaloacetate inverted as a result of its equilibration with fumarate, a symmetrical molecule (see also Table I), and where as mentioned above (Pyr \ text{OAA}) = (OAA \ text{PEP}) \ (Pyr \ text{PEP}) \ (Pyr \ text{OAA}) (see also Fig. 2 and Table I).

\[ [\text{C}_3\text{OAA}]^{\text{Glu}} = X \cdot \alpha\text{KG} \cdot (\alpha\text{KG} \rightarrow \text{OAA}) / 2 \]

\[ + (\text{OAA}) \cdot (\text{Pyr} \ | \text{OAA}) \cdot [\text{C}_3\text{OAA}]^{\text{Glu}} = (1 - [\text{OAA}]) \cdot (\text{Pyr} \ | \text{OAA}) \cdot [\text{C}_3\text{OAA}]^{\text{Glu}} \]

Then, \( [\text{C}_3\text{OAA}]^{\text{Glu}} = X \cdot \alpha\text{KG} \cdot (\alpha\text{KG} \rightarrow \text{OAA}) / 2 \cdot (1 - [\text{OAA}]) \cdot (\text{Pyr} \ | \text{OAA}) \cdot [\text{C}_3\text{OAA}]^{\text{Glu}} \)
And, \([C_1 + C_3\text{OAA}]^{\text{C}\text{Glu}} = X\cdot[\text{Glu} \rightarrow \text{aKG}]\cdot(\text{aKG} \rightarrow \text{OAA})\cdot[1 + [\text{OAA}]_2]/(1 - [1 - (\text{OAA})] \cdot (\text{Pyr} \% \text{ OAA})].\)

From Fig. 4 one can also deduce that

\[
[C_1\text{OAA}]^{\text{C}\text{Glu}} = ([C_2\text{OAA}]^{\text{C}\text{Glu}} \cdot \frac{[\text{AcCoA} \% \text{ OAA}]/2}{[\text{C}1\text{OAA}]^{\text{C}\text{Glu}}}) + \frac{[\text{TCA}]_2/2}{\sum_{n=0}^{\infty} [1 - (\text{OAA})] \cdot (\text{Pyr} \% \text{ OAA})} \cdot (\text{TCA} \% 2)\quad (\text{Eq. 13})
\]

where, as mentioned in Equation 9 (\text{AcCoA} \% \text{ OAA}) = (\text{OAA} \rightarrow \text{PEP}) \cdot (\text{PEP} \rightarrow \text{Pyr}) \cdot (\text{Pyr} \rightarrow \text{AcCoA})\cdot[\text{C}\text{1\text{OAA}}]^{\text{C}\text{Glu}}/(\text{[TCA]}_2/2)\). Represent the \(C1\text{OAA}\) formed directly from the C-2 and C-3 of oxaloacetate.

Fig. 6 shows that

\[
[C_2 + C_3\text{OAA}]^{\text{C}\text{Glu}} = X \cdot ([\text{Glu} \rightarrow \text{aKG}] \cdot (\text{aKG} \rightarrow \text{OAA}) \cdot \sum_{n=0}^{\infty} ([\text{Pyr} \% \text{ OAA}] + [\text{TCA}]_2) + (\text{AcCoA} \% \text{ OAA})/2 + (\text{C}2\text{OAA}]^{\text{C}\text{Glu}})/(\text{TCA} \% 2)\). \quad (\text{Eq. 14})
\]

From Fig. 4, one can deduce that the C-3 of glutamate yields equal amounts of C-2 and C-3 of oxaloacetate, so that

\[
[C_2\text{OAA}]^{\text{C}\text{Glu}} = [C_3\text{OAA}]^{\text{C}\text{Glu}} = X \cdot ([\text{Glu} \rightarrow \text{aKG}] \cdot (\text{aKG} \rightarrow \text{OAA}) \cdot (1/2)/(1 - (\text{Pyr} \% \text{ OAA})) - ([\text{TCA}]_2) + (\text{AcCoA} \% \text{ OAA})/2) \quad (\text{Eq. 15})
\]

Using Equation 14, Equation 13 can be rewritten as

\[
[X \cdot ([\text{Glu} \rightarrow \text{aKG}] \cdot (\text{aKG} \rightarrow \text{OAA}) \cdot ([\text{TCA}]_2) + (\text{AcCoA} \% \text{ OAA}))/2 - (\text{Pyr} \% \text{ OAA})/(1 - (\text{OAA})], (\text{Pyr} \% \text{ OAA}))/2] \quad (\text{Eq. 19})
\]
Therefore,
\[
[C_3\text{OAA}]^{\text{C-Glu}}/[C_3\text{OAA}]^{\text{Glu}} = \left(\frac{[\text{TCA}]}{[\text{OAA}]} + \frac{[\text{AcCoA}]}{[\text{OAA}]}/2\right)/(1 - \frac{[\text{OAA}]^\circ}{[\text{OAA}]}) - \frac{[\text{TCA}]}{[\text{OAA}]} - \frac{[\text{AcCoA}]}{[\text{OAA}]}/2
\] (Eq. 20)

Let us call A the Laffer Ratio, then one can deduce from Fig. 4 that
\[
[C_3\text{OAA}]^{\text{C-Glu}}/[C_3\text{OAA}]^{\text{Glu}} = \left(\frac{[\text{Glu}]}{[\text{Glc}]}\right)^2[C_3\text{Glu}]^{\text{C-Glu}}/\left[C_3\text{Ala}\right]^{\text{C-Glu}} = A
\] (Eq. 21)

and
\[
[\text{CO}_2]^{\text{C-Glu}}/[\text{CO}_2]^{\text{Glu}} = \left[\frac{\text{C}_3\text{Ala}}{\text{C}_3\text{Glu}}\right] \left[C_3\text{Ala}/C_3\text{Glu}\right] = B
\] (Eq. 23)

where the value B of the latter 2 ratios can be calculated from Equations 11 and 15: \(B = 1 - (\text{Pyr} \parallel \text{OAA}) + (\text{OAA} \parallel \text{Pyr})/\text{OAA})/(1 - (\text{Pyr} \parallel \text{OAA}) - ([\text{TCA}] \parallel + (\text{AcCoA} \parallel \text{OAA})/2).\)

Combining Equations 21 and 23, yields \((A + 1)/(B - A - 1)\)

\[1 - (\text{Pyr} \parallel \text{OAA})] - \frac{[\text{OAA}]}{[\text{OAA}]} + (\text{Pyr} \parallel \text{OAA})\]

\[\text{Let C} = \left(\frac{1}{\text{Pyr} \parallel \text{OAA}}\right) - \frac{[\text{OAA}]}{[\text{OAA}]} - (\text{Pyr} \parallel \text{OAA})\]

\[\text{Let D} = \left[C_3\text{Glu}\right]^{\text{C-Glu}}/[C_3\text{Ala}]^{\text{C-Glu}} = \frac{[\text{OAA}]}{\text{OAA}}
\]

From Fig. 4 and Equations 11 and 12, one can deduce that
\[D = \left(1 - \frac{2}{\text{OAA}}\right) \left(\frac{\text{Pyr} \parallel \text{OAA}}{[\text{OAA}]}\right) - \frac{[\text{TCA}]}{[\text{OAA}]} - (\text{Pyr} \parallel \text{OAA})
\] \(\text{TCA})(\text{OAA})/\text{OAA})/\text{OAA}) - (\text{Pyr} \parallel \text{OAA}) - (\text{OAA} \parallel \text{Pyr})/\text{OAA})/\text{OAA})\]

\[\text{Let C} = \left(\frac{1}{\text{Pyr} \parallel \text{OAA}}\right) - \frac{[\text{OAA}]}{[\text{OAA}]} - (\text{Pyr} \parallel \text{OAA})\]

\[\text{Let D} = \left[C_3\text{Glu}\right]^{\text{C-Glu}}/[C_3\text{Ala}]^{\text{C-Glu}} = \frac{[\text{OAA}]}{\text{OAA}}
\]

\[\text{Let E} = \left[C_3\text{Ala}\right]^{\text{C-Glu}}/\left[C_3\text{Glu}\right]^{\text{C-Glu}}\]

\[\text{Combining the latter equation and Equation 24, we obtain}
\]

\[\left[C_3\text{Glu}\right]^{\text{Glu}}/\left[C_3\text{Glu}\right]^{\text{Glu}} = \left(C_3\text{Ala}\right)\left(C_3\text{Ala}\right)\left(\text{Pyr} \parallel \text{OAA}) + \left(\frac{[\text{OAA}]}{[\text{OAA}]}\right)\left(\text{Pyr} \parallel \text{OAA})\right)\left(1 - \frac{[\text{OAA}]}{[\text{OAA}]} - (\text{Pyr} \parallel \text{OAA})\right)
\] (Eq. 25)

\[\text{Let us call E} = \left[C_3\text{Glx}\right]^{\text{Glu}}/\left[C_3\text{Glx}\right]^{\text{Glu}} = \left[C_3\text{Ala}\right]^{\text{C-Glu}}/\left[C_3\text{Glu}\right]^{\text{C-Glu}}
\]

\[\text{Combining Equations 24 and 26, we obtain}
\]

\[\frac{1}{\left(1 - \frac{\text{Pyr} \parallel \text{OAA}}{\text{OAA}}\right) + 2 \left(\text{Pyr} \parallel \text{OAA}) - 1\right)} = \left(\frac{\text{TCA} \parallel}}{\text{Pyr} \parallel \text{OAA})\right) - (\text{Pyr} \parallel \text{OAA})\right)
\] (Eq. 27)

\[\text{Let us call F} = \frac{G}{D/E}
\]

\[\text{F} = \left(1 - \frac{1}{2}\text{ (OAA)}\right) \left(\text{Pyr} \parallel \text{OAA})\right)\left(1 - \frac{\text{Pyr} \parallel \text{OAA}}{\text{OAA})\right) + 2 \left(\text{Pyr} \parallel \text{OAA}) - 1\right) = (2 + C) \left(\text{OAA})/\text{OAA}\right)\left(\text{Pyr} \parallel \text{OAA})\right)\left(1 - \frac{\text{OAA}]}{[\text{OAA}]}\right)
\] (Eq. 29)

\[\text{Therefore, i} = \left(1 + C\right)\left(2 + C\cdot(1 + F)\right)
\]

\[
\text{Then, } [\text{C}_3\text{OAA}]^{\text{C-Glu}}/[\text{C}_3\text{OAA}]^{\text{Glu}} = \left(\frac{[\text{TCA}]}{[\text{OAA}]} + \frac{[\text{AcCoA}]}{[\text{OAA}]}/2\right)/(1 - \frac{[\text{OAA}]}{[\text{OAA}])} - \frac{[\text{TCA}]}{[\text{OAA}]} - \frac{[\text{AcCoA}]}{[\text{OAA}]}/2
\]

\[\text{Let us call G} = \frac{1}{[\text{Pyr} \parallel \text{OAA})]/\left([\text{OAA}]\right)\left(\text{Pyr} \parallel \text{OAA})\right)\left(1 + \text{G}\right)
\]

\[\text{and}
\]

\[
\text{Eq. 31}
\]

\[\text{From Fig. 6, it can be deduced that}
\]

\[\text{Eq. 32}
\]

\[\text{where}
\]

\[\text{Eq. 33}
\]

\[\text{And Fig. 4 shows that}
\]

\[\text{Eq. 34}
\]

\[\text{where (Pyr} \parallel \text{Lac}) \text{ is the proportion of pyruvate directly converted to lactate.}
\]

\[\text{Combining Equations 32 and 34, it follows that}
\]

\[\text{Eq. 35}
\]

\[\text{Then, } [\text{C}_3\text{OAA}]^{\text{C-Glu}}/\text{[C}_3\text{OAA}]^{\text{C-Glu}} \text{ can be calculated:}
\]

\[\text{Eq. 36}
\]

\[\text{and similarly,}
\]

\[\text{Eq. 37}
\]

\[\text{Furthermore, } [\text{C}_3\text{OAA}]^{\text{C-Glu}}/\text{[C}_3\text{OAA}]^{\text{C-Glu}} \text{ are theoretically identical (see Figs. 4 and 6).}
\]

\[\text{The proportion } \text{(OAA} \parallel \text{Asp}) \text{ of oxaloacetate directly converted into aspartate is given by (OAA} \parallel \text{Asp}) = \left[C_3\text{Asp}\right]^{\text{C-Glu}}/\left[C_3\text{OAA}]^{\text{C-Glu}} \text{ (see Fig. 4).}
\]

\[\text{From the latter equation and Equation 37, it follows that}
\]

\[\text{Eq. 38}
\]

\[\text{From Figs. 2 and 4, it can be deduced that}
\]

\[\text{Eq. 39}
\]

\[\text{Thus, (TCA} \parallel \text{) and (AcCoA} \parallel \text{OAA} \text{ can be calculated from}
\]
From Equations 14 and 33, it follows that
\[
\text{OAA} \rightarrow \text{Pyr} \rightarrow \text{Pyr} \rightarrow \text{AcCoA} \rightarrow \text{PEP} \rightarrow \text{TCA} \rightarrow \text{Glu} + \text{Gln}
\]
where, as indicated above, (Pyr + Acetyl-CoA) with the proportion (Pyr + Acetyl-CoA) converted to 3-phosphoglycerate.

From Equations 38 and 39, and knowing (AcCoA + OAA)/[TCA] and [OAA → Cit] + (OAA → PEP), we obtain:
\[
[\text{AcCoA} + \text{OAA}]/[\text{TCA}] \cdot [1 - \text{OAA} \rightarrow \text{Asp}] = (\text{OAA} \rightarrow \text{PEP} \rightarrow \text{TCA}) \cdot [\text{PEP} \rightarrow \text{TCA}]
\]
Equations 38 and 43, one can deduce that
\[
\text{OAA} \rightarrow \text{PEP} = (\text{Pyr} \rightarrow \text{OAA}) + (\text{OAA} \rightarrow \text{PEP}) \cdot (\text{Pyr} \rightarrow \text{Lac}) + (\text{OAA} \rightarrow \text{PEP}) \cdot (\text{Pyr} \rightarrow \text{Ala}) + (\text{AcCoA} \rightarrow \text{OAA}) \cdot [1 - \text{OAA} \rightarrow \text{Asp}] + (\text{OAA} \rightarrow \text{PEP}) \cdot (\text{PEP} \rightarrow \text{TCA})
\]
where, as indicated above, (Pyr + OAA) = (OAA → PEP). Then (PEP → 3PG) = (OAA → PEP → 3PG)/OAA → PEP, and since (PEP → PEP) + (PEP → 3PG) = 1, (PEP → PEP) = 1 – (PEP → 3PG).

The parameter (OAA → Cit) can be calculated from Equation 38:
\[
\text{OAA} \rightarrow \text{Cit} = [1 - \text{OAA} \rightarrow \text{Asp}] - \text{OAA} \rightarrow \text{PEP}
\]
Since the recycling factor in the tricarboxylic acid cycle (TCA), which accounts also for α-ketoglutarate recycling through glutamate and glutamine, is equal to (OAA → Cit)/[AcCoA/KG → OAA] (see Equation 8), it can be calculated that
\[
\text{AcCoA} \rightarrow \text{OAA} = (\text{TCA} + \text{Cit}) / \text{OAA} \rightarrow \text{Cit}
\]
From Equation 7 and since as shown in Fig. 1, (Glu → aKG) + (Glu → TCA) + (Glu → Gln) = 1 (see Equation 4), it follows that
\[
\text{Glu} \rightarrow \text{aKG} + \text{Glu} \rightarrow \text{TCA} + \text{Glu} \rightarrow \text{Gln}
\]
Therefore, the α-ketoglutarate recycling through glutamate and glutamine (\text{aKG} + \text{Glu} + \text{Gln}) is equal to \text{aKG} + \text{Glu} + \text{Gln} = \text{cTCA/KG → OAA}. (\text{aKG} → \text{OAA}).

Then, (\text{Glu} → \text{aKG}) can be calculated from Equations 10 and 50. Equation 39 yields (OAA → PEP) + (PEP → PEP) + (Pyr → AcCoA) = (\text{AcCoA} + \text{OAA}/[\text{TCA} + \text{Cit}])/(OAA → Cit).

The proportion (Pyr → AcCoA) of pyruvate converted into acetyl-CoA is given by (Pyr → AcCoA) = (OAA → PEP + PEP + PEP + PEP)/OAA → PEP. Similarly, the proportions (Pyr → Lac), (Pyr → Ala), and (Pyr → OAA) corresponding to the proportions of pyruvate transformed into lactate, alanine, and oxaloacetate, respectively, are obtained as follows. (Pyr → Lac) = (OAA → PEP) + (Pyr → PEP) + (Pyr → Lac)/[OAA → PEP + PEP + PEP + PEP + PEP]; (Pyr → Ala) = (OAA → PEP + PEP + PEP + PEP + PEP)/[OAA → PEP + PEP + PEP + PEP + PEP]; (Pyr → OAA) = (Pyr + OAA)/[OAA → OAA + PEP + PEP + PEP + PEP + PEP]; where (Pyr + OAA) = (OAA → PEP + PEP + PEP + PEP + PEP)/OAA → OAA.

The proportion (3PG → Glc) of 3-phosphoglycerate which yields glucose is given by (3PG → Glc) = J/(J + L) (see also Fig. 4).

Calculations of the Enzymatic Fluxes—It should be stressed that, in this study, we did not calculate enzyme activities. Our model allowed us to calculate only mean fluxes in relation to glutamate metabolism. In this model, as already mentioned, a flux through a given enzyme is taken as the formation of one product per g dry wt and per unit of time (1 h in this study) of the reaction catalyzed by this enzyme. It should be pointed out that oxaloacetate is the only metabolite common to three of the metabolic cycles involved in glutamate 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 glutamate metabolism (noted [OAA] in Fig. 3), these oxaloacetate molecules containing 1, 2, 3, 4, or 0 carbon atoms derived from glutamate. Fig. 3 gives a schematic representation providing the basic elements needed for such a determination. In the left panel of Fig. 3, which is derived from Fig. 2 (Pyr + OAA), (TCA + Cit) and (AcCoA + OAA) represent the oxaloacetate recycled in the “OAA → PEP → Pyr → OAA,” the tricarboxylic acid and the “OAA → PEP → Pyr → AcCoA → Cit” cycles, respectively (see Fig. 2 and Table 1).

It should be stressed that the proportions (TCA + Cit) and (OAA + AcCoA) take also into account the recycling in the “Glu → aKG → Glu” and “Glu → Glu → Gln” cycles. Let us call (AcCoA + OAA) the proportion of the acetyl-CoA molecules derived from glutamate that have been condensed with oxaloacetate molecules of endogenous origin to give citrate. It is necessary to introduce this proportion (see Fig. 2) to calculate correctly the oxaloacetate formation from glutamate by avoiding to take into account twice the citrate.
molecules synthesized from an oxaloacetate and an acetyl-CoA molecules originating both from glutamate. The proportion \((AcCoA + OAA \rightarrow Cit)\) also allows one to take into account the citrate molecules formed from an acetyl-CoA molecule derived from glutamate and an oxaloacetate molecule arising from endogenous substrates. Thus, the proportion of acetyl-CoA derived from glutamate and condensed with endogenous oxaloacetate to give oxaloacetate via the tricarboxylic acid cycle is equal to \((AcCoA + OAA \rightarrow Cit)\) (Eq. 2 and 3). Then, at each turn of the multicycle, the additional proportion of oxaloacetate formed as a result of the operation of the “OAA \(\rightarrow\) PEP \(\rightarrow\) Pyr \(\rightarrow\) AcCoA \(\rightarrow\) Cit \(\rightarrow\) OAA” cycle is \((AcCoA + OAA \rightarrow Cit)\) (Eq. 4), while the proportion of oxaloacetate formed by the “OAA \(\rightarrow\) PEP \(\rightarrow\) Pyr \(\rightarrow\) OAA” cycle and by the tricarboxylic acid cycle are \((Pyr \| OAA)\) and \((TCA\|)\), respectively.

The right panel of Fig. 3 summarizes the oxaloacetate formation from glutamate shown in more detail in the left panel of the same figure. It allows us to calculate the total amount of oxaloacetate derived from glutamate \((OAA(Glu))\) using the following equations derived from Fig. 3, in which the repetitiveness of the formation of oxaloacetate by the operation of the multicycle is taken into account thanks to the parameter \((Pyr \| OAA)\) + \((TCA\|)\) + \((AcCoA + OAA \rightarrow Cit)\) (Eq. 4), which represents the proportion of oxaloacetate recycled at each turn of the multicycle presented in Fig. 2.

\[
\begin{align*}
\text{[OAA]}^{Glu} = & X \cdot (\text{[Glu} \rightarrow \alpha KG] \cdot (\alpha KG \rightarrow OAA) \cdot \sum_{n=0}^{\infty} \text{[Pyr} \| OAA]) \nonumber \\
+ & (TCA\|) + (AcCoA + OAA \rightarrow Cit) \cdot (AcCoA \| OAA)^n
\end{align*}
\]

Then

\[
\begin{align*}
\text{[OAA]}^{Glu} = & X \cdot (\text{[Glu} \rightarrow \alpha KG] \cdot (\alpha KG \rightarrow OAA) + 1 - \text{[Pyr} \| OAA]) \\
- & (TCA\|) - (AcCoA + OAA \rightarrow Cit) \cdot (AcCoA \| OAA)
\end{align*}
\]

In the latter equation, \(1/[1 - \text{[Pyr} \| OAA] - (TCA) - (AcCoA + OAA \rightarrow Cit) \cdot (AcCoA \| OAA)]\) represents the proportion of the oxaloacetate formation over an infinite number of multicycle turns, i.e. the oxaloacetate turnover. Using the NMR data obtained with \(3^{-13}C\)glutamate as substrate, the value of \((AcCoA + OAA \rightarrow Cit)\) can be obtained by calculating first \(1 - (AcCoA + OAA \rightarrow Cit)\), which represents the proportion of acetyl-CoA molecules derived from added glutamate (noted \([AcCoA]^{Glu}\)) that has been condensed with oxaloacetate molecules also derived from added glutamate (noted \([OAA]^{Glu}\)).

This proportion can be assessed by the ratio of \(2^{-13}C\)Acetyl-CoA, which condenses with \(2^{-13}C\)oxaloacetate. This ratio, reflected by the proportion of the C-4 and C-3 of glutamate plus \((AcCoA + OAA \rightarrow Cit)\) (Eq. 53), found to be coupled on the NMR spectra, was corrected to take into account the total oxaloacetate formation from glutamate. Thus,

\[
\begin{align*}
1 - (AcCoA + OAA \rightarrow Cit) = & [OAA]^{Glu} \cdot (OAA \rightarrow Cit) \cdot [1 \\
- & (\alpha KG \rightarrow OAA)] \cdot (\text{[Glu} \rightarrow \alpha KG]) \cdot (\alpha KG \rightarrow OAA) \cdot \text{[Glu} \rightarrow \alpha KG]
\end{align*}
\]

(Eq. 53)

Since, as mentioned above, the C-3 and C-2 of Glx are formed in equal amounts from \(3^{-13}C\)Glu (see Fig. 4), it follows that:

\[
\begin{align*}
1 - (AcCoA + OAA \rightarrow Cit) = & [OAA]^{Glu} \cdot (OAA \rightarrow Cit) \cdot [1 \\
- & (\alpha KG \rightarrow OAA)] \cdot (\text{[Glu} \rightarrow \alpha KG]) \cdot (\alpha KG \rightarrow OAA) \cdot \text{[Glu} \rightarrow \alpha KG]
\end{align*}
\]

(Eq. 54)

Similarly, one can demonstrate that

\[
\begin{align*}
1 - (AcCoA + OAA \rightarrow Cit) = & [OAA]^{Glu} \cdot (OAA \rightarrow Cit) \cdot [1 \\
- & (\alpha KG \rightarrow OAA)] \cdot (\text{[Glu} \rightarrow \alpha KG]) \cdot (\alpha KG \rightarrow OAA) \cdot \text{[Glu} \rightarrow \alpha KG]
\end{align*}
\]

(Eq. 55)

where \((OAA + AcCoA \rightarrow Cit)\) is the proportion of oxaloacetate molecules derived from added glutamate that have been condensed with acetyl-CoA molecules not derived from added glutamate.

Finally, the latter equation together with Equations 39 and 54 yield

\[
\begin{align*}
(OAA + AcCoA \rightarrow Cit) = & 1 - \\
\begin{align*}
1 - (AcCoA + OAA \rightarrow Cit) \cdot (AcCoA \| OAA)/(TCA\|)
\end{align*}
\end{align*}
\]

(Eq. 56)

It is possible to determine the amount of oxaloacetate, \([OAA]^{non-Glu}\), and acetyl-CoA, \([AcCoA]^{non-Glu}\), not derived from added glutamate that condense with glutamate-derived acetyl-CoA and glutamate-derived oxaloacetate, respectively.

\[
\begin{align*}
\text{[OAA]}^{non-Glu} = & \text{[AcCoA]}^{Glu} \cdot (AcCoA + OAA \rightarrow Cit) = [OAA]^{Glu}
\end{align*}
\]

(Eq. 57)

and

\[
\begin{align*}
\text{[AcCoA]}^{non-Glu} = & [OAA]^{Glu} \cdot (OAA \rightarrow Cit) \cdot (AcCoA + OAA \rightarrow Cit)
\end{align*}
\]

(Eq. 58)

In view of the fact that \([OAA]^{Glu}\) and \((AcCoA + OAA \rightarrow Cit)\) 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 (2), one can write that \([OAA]^{Glu} = \text{flux through pyruvate carboxylase} + \text{flux through } \alpha\text{-keto glutarate dehydrogenase}\).

To determine correctly enzymatic fluxes during glutamate metabolism, one should know the total amount of glutamate involved in this process, noted \([Glu]^{Glu}\), which, as indicated in Figs. 1 and 2, has two possible origins.

(i) The glutamate noted \(\text{[Glu]}^{Glu}\) is derived directly from the glutamate utilized which undergoes a recycling through \(\alpha\)-ketoglutarate and glutamine: \(\text{Glu} \rightarrow [Glu]^{Glu} = \text{glutamate utilized} \cdot \text{recycling ratio of glutamate through } \alpha\text{-ketoglutarate and glutamine}\). From Equation 5, it follows that

\[
\begin{align*}
\text{Glu} \rightarrow [Glu]^{Glu} = & X \cdot [Glu \| \alpha KG + Gln]
\end{align*}
\]

(Eq. 59)

(ii) The glutamate noted \(\text{[Cit} \rightarrow \text{[Glu]}^{Glu}\) is formed from molecules of \(\alpha\)-ketoglutarate or glutamine which are derived from citrate molecules:

\[
\begin{align*}
\text{Cit} \rightarrow [Glu]^{Glu} = & \text{Cit} \rightarrow [\alpha KG]^{Glu} \cdot (\alpha KG \rightarrow Gln)
\end{align*}
\]

(Eq. 60)

where \(\text{Cit} \rightarrow [Gln]^{Glu} = \text{Cit} \rightarrow [Glu]^{Glu}\). From (Eq. 1 and 2).

Combining the latter two equations, we obtain

\[
\begin{align*}
\text{Cit} \rightarrow [Glu]^{Glu} = & \text{Cit} \rightarrow [Glu]^{Glu} + \text{Cit} \rightarrow [Gln]^{Glu}
\end{align*}
\]

(Eq. 61)

Replacing \((\text{Glu} \rightarrow \text{Glu})\) by \((\text{Glu} \| \text{Gln})\), as indicated in Equation 3, it follows that:

\[
\begin{align*}
\text{Cit} \rightarrow [\alpha KG]^{Glu} = & \text{Cit} \rightarrow [\alpha KG]^{Glu} \cdot (\alpha KG \rightarrow Gln) [1 - (\text{Glu} \| \text{Gln})]
\end{align*}
\]

(Eq. 62)

where \(\text{Cit} \rightarrow [\alpha KG]^{Glu} = \text{[Cit formed} \cdot \text{recycling ratio of } \alpha\text{-ketoglutarate through glutamate and glutamine}\). From Equation 6 one can calculate that

\[
\begin{align*}
\text{Cit} \rightarrow [\alpha KG]^{Glu} = & [\text{Cit}]^{Glu} \cdot [1 - (\text{Glu} \| \text{Gln})] [1 - (\text{Glu} \| \alpha KG)]
\end{align*}
\]

(Eq. 63)
Since \( [\text{Glu}]_0 + \alpha \text{KG} + \text{Gln} ] = 1/1 - (\text{Glu}]_0 + \alpha \text{KG} - (\text{Glu}]_0 + \text{Gln}]) \) (see Equation 5), one can deduce from Equations 62 and 63 that

\[
 [\text{Cit}]_{[\text{Glu}]} \cdot (\alpha \text{KG} \rightarrow \text{Glu}) = [X - (\text{Glu} \| \text{Gln}) \] and 
\[
 (\text{Glu} \| \text{Gln}) = [\text{Cit}]_{[\text{Glu}]} \cdot (\text{Glu} \| \alpha \text{KG} + \text{Gln}) + (\text{Glu} \| \alpha \text{KG} + \text{Gln}) \] (Eq. 64)
\]

The total amount of glutamate involved in the metabolism is obtained from Equations 59 and 64

\[
 [\text{Glu}]_{[\text{Glu}]} = [\text{Glu}]_{[\text{Glu}]}^{\text{Glu}} + [\text{Cit}]_{[\text{Glu}]} \cdot X \cdot (\text{Glu} \rightarrow \alpha \text{KG}) + [\text{Cit}]_{[\text{Glu}]} \cdot (\text{Glu} \| \alpha \text{KG} + \text{Gln}) \] (Eq. 65) but it cannot be calculated because \( X \cdot (\text{Glu} \| \alpha \text{KG} + \text{Gln}) \) and \( (\text{Glu} \| \alpha \text{KG} + \text{Gln}) \) cannot be derived from the labeled carbon data.

The total amount of \( \alpha \text{-ketoglutarate} \) formed during glutamate metabolism is obtained from the sum of the \( \alpha \text{-ketoglutarate} \) formed directly from the glutamate utilized, \( \text{Glu} \rightarrow \alpha \text{KG}[^{\text{Glu}}] \) (see Equation 59), and the derived from citrate, \( \text{Cit} \rightarrow \alpha \text{KG}[^{\text{Cit}}] \) (see Equation 63) (see also Figs. 2 and 4).

\[
 [\text{Glu} \rightarrow \alpha \text{KG}] = [\text{Glu}]_{[\text{Glu}]}^{\text{Glu}} + [\text{Cit}]_{[\text{Glu}]} \cdot (\text{Glu} \| \alpha \text{KG} + \text{Gln}) \] (Eq. 66)

where \( \text{Cit} \rightarrow \alpha \text{KG}^{[\text{Cit}} = \text{Glu} \rightarrow \text{Glu}^{[\text{Glu}]} + \text{Glu}^{[\text{Glu}]} \rightarrow \alpha \text{KG} \) and \( \text{Glu} \rightarrow \alpha \text{KG} \) cannot be derived from the labeled carbon data.

From Equation 66 and Fig. 2, unidirectional flux of glutamate to \( \alpha \text{-ketoglutarate} \), noted \( [\text{Glu} \rightarrow \alpha \text{KG}] \), can be calculated as follows:

\[
 (\text{Glu} \rightarrow \alpha \text{KG}) = [\text{Glu}]_{[\text{Glu}]}^{\text{Glu}} + [\text{Cit}]_{[\text{Glu}]} \cdot \text{Glu} + \alpha \text{KG} + \text{Gln} \] (Eq. 67) and

\[
 \text{net}[\text{Glu} \rightarrow \alpha \text{KG}] = (\text{Glu} \rightarrow \alpha \text{KG}) - [\text{Glu} \rightarrow \alpha \text{KG}] = [X - (\text{Glu} \rightarrow \text{Cit})] + [\text{Cit}]_{[\text{Glu}]} \cdot (\text{Glu} \| \alpha \text{KG} + \text{Gln}) \] (Eq. 68) where \( [\text{Cit}]_{[\text{Glu}]} \) is the proportion of citrate-derived \( \alpha \text{-ketoglutarate} \) converted to glutamate which takes into account the total recycling through the \( \text{Glu} \rightarrow \alpha \text{KG} \rightarrow \text{Glu} \) and \( \text{Glu} \rightarrow \alpha \text{KG} \rightarrow \text{Glu} \) cycles (see under “Notation”).

Thus, net flux of glutamate to \( \alpha \text{-ketoglutarate} \), noted \( \text{net}[\text{Glu} \rightarrow \alpha \text{KG}] \), can be obtained from the two latter equations:

\[
 \text{net}[\text{Glu} \rightarrow \alpha \text{KG}] = (\text{Glu} \rightarrow \alpha \text{KG}) - (\text{Glu} \rightarrow \alpha \text{KG}) = [X - (\text{Glu} \rightarrow \text{Cit})] + [\text{Cit}]_{[\text{Glu}]} \cdot \text{Glu} + \alpha \text{KG} + \alpha \text{KG} + \text{Gln} \] (Eq. 69) where, as mentioned above, \( (\text{Glu} \rightarrow \alpha \text{KG}) = \alpha \text{KG} \rightarrow \text{Glu} \) and \( \alpha \text{KG} + \text{Gln} \) are equal to \( 1/1 - (\text{Glu} \| \alpha \text{KG} + \text{Gln} + \text{Gln} = (\text{Glu} \| \alpha \text{KG}) - (\text{Glu} \| \alpha \text{KG} + \text{Gln}) \), and \( (\text{Glu} \| \alpha \text{KG}) + \text{Gln} \) (Equations 5 and 6), and since, from Equations 2 and 3 (\( \alpha \text{KG} \rightarrow \text{Glu} \) and \( \text{Glu} \| \alpha \text{KG} \) + \( \text{Glu} \| \alpha \text{KG} + \text{Gln} \) (Equations 5 and 6), and since, from Equations 2 and 3 (\( \alpha \text{KG} \rightarrow \text{Glu} \) and \( \text{Glu} \| \alpha \text{KG} \) + \( \text{Glu} \| \alpha \text{KG} + \text{Gln} \) (Equations 5 and 6), and since, from Equations 2 and 3 (\( \alpha \text{KG} \rightarrow \text{Glu} \) and \( \text{Glu} \| \alpha \text{KG} \) + \( \text{Glu} \| \alpha \text{KG} + \text{Gln} \) (Equations 5 and 6), and since, from Equations 2 and 3 (\( \alpha \text{KG} \rightarrow \text{Glu} \) and \( \text{Glu} \| \alpha \text{KG} \) + \( \text{Glu} \| \alpha \text{KG} + \text{Gln} \) (Equations 5 and 6), and since, from Equations 2 and 3 (\( \alpha \text{KG} \rightarrow \text{Glu} \) and \( \text{Glu} \| \alpha \text{KG} \) + \( \text{Glu} \| \alpha \text{KG} + \text{Gln} \) (Equations 5 and 6), and since, from Equations 2 and 3 (\( \alpha \text{KG} \rightarrow \text{Glu} \) and \( \text{Glu} \| \alpha \text{KG} \) + \( \text{Glu} \| \alpha \text{KG} + \text{Gln} \) (Equations 5 and 6), and since, from Equations 2 and 3 (\( \alpha \text{KG} \rightarrow \text{Glu} \) and \( \text{Glu} \| \alpha \text{KG} \) + \( \text{Glu} \| \alpha \text{KG} + \text{Gln} \) (Eq. 69) where, as mentioned above, \( (\text{Glu} \rightarrow \alpha \text{KG}) = \alpha \text{KG} \rightarrow \text{Glu} \) and \( \alpha \text{KG} + \text{Gln} \) are equal to \( 1/1 - (\text{Glu} \| \alpha \text{KG} + \text{Gln} + \text{Gln} = (\text{Glu} \| \alpha \text{KG}) - (\text{Glu} \| \alpha \text{KG} + \text{Gln}) \), and \( (\text{Glu} \| \alpha \text{KG}) + \text{Gln} \) (Equations 5 and 6), and since, from Equations 2 and 3 (\( \alpha \text{KG} \rightarrow \text{Glu} \) and \( \text{Glu} \| \alpha \text{KG} \) + \( \text{Glu} \| \alpha \text{KG} + \text{Gln} \) (Eq. 69)
Model of Glutamate Metabolism

\[ \text{[Glnase]} = (X \cdot (\text{Cit}^{\text{Glu}} \cdot (\alphaKG \rightarrow \text{Glu}) \cdot ([\text{Glu} \rightarrow \text{Gln}] \cdot (\text{Gln} \rightarrow \text{Glu}) \quad \text{(Eq. 80)} \]

but, these fluxes cannot be calculated since labeled carbon data don't allow us to obtain the value of \([\text{Glu} \rightarrow \text{Gln}].\)

Flux through pyruvate dehydrogenase, which is equal to [\text{AcCoA}^{\text{Glu}}], can be derived from Fig. 2: [\text{AcCoA}^{\text{Glu}} = [\text{OAA}^{\text{Glu}} \cdot (\text{OAA} \rightarrow \text{PEP} \cdot (\text{PEP} \rightarrow \text{Pyr}) \cdot (\text{Pyr} \rightarrow \text{AcCoA}).]

Flux through phosphoenolpyruvate carboxykinase is given by [\text{OAA}^{\text{Glu}} \cdot (\text{OAA} \rightarrow \text{PEP}) \cdot (\text{PEP} \rightarrow \text{Pyr}) \cdot (\text{Pyr} \rightarrow \text{AcCoA}).]

Flux through pyruvate kinase is equal to [\text{OAA}^{\text{Glu}} \cdot (\text{OAA} \rightarrow \text{PEP}) \cdot (\text{PEP} \rightarrow \text{Pyr}) \cdot (\text{Pyr} \rightarrow \text{AcCoA}).]

Flux through lactate dehydrogenase is obtained by multiplying flux through pyruvate kinase by (\text{Pyr} \rightarrow \text{Lac}).

Flux through phosphoglyceromutase is given by [\text{Glc}^{\text{Glu}} \cdot (\text{Glc} \rightarrow \text{PEP}) \cdot (\text{PEP} \rightarrow \text{Pyr}) \cdot (\text{Pyr} \rightarrow \text{AcCoA}).]

Flux through glucose-6-phosphatase is equal to [\text{Glc}^{\text{Glu}} \cdot (\text{Glc} \rightarrow \text{PEP}) \cdot (\text{PEP} \rightarrow \text{3PG} \cdot (\text{3PG} \rightarrow \text{Glc}) \cdot \text{corresponds to 2} \cdot \text{(Glc)} \cdot \text{since fluxes are expressed in C3 units.}]

Flux through citrate synthase, [\text{CS}], is given by the amount of citrate formed as shown in Fig. 2 and Equation 75.

Net flux through 3-phosphoglycerate dehydrogenase and transaminases resulting in a net conversion of glutamate into \(\alpha\)-ketoglutarate is given by the sum of the net transaminase fluxes involved in alanine, aspartate, and serine formation.

Net flux through alanine aminotransferase is equal to alanine accumulation: \([\text{Ala}]^{\text{Glu}} = [\text{OAA}^{\text{Glu}} \cdot (\text{OAA} \rightarrow \text{PEP}) \cdot (\text{PEP} \rightarrow \text{Pyr}) \cdot (\text{Pyr} \rightarrow \text{Ala}).]

Net flux through aspartate aminotransferase is equal to aspartate accumulation: \([\text{Asp}]^{\text{Glu}} = [\text{OAA}^{\text{Glu}} \cdot (\text{OAA} \rightarrow \text{PEP}) \cdot (\text{PEP} \rightarrow \text{3PG} \cdot (\text{3PG} \rightarrow \text{Glc}) \cdot \text{corresponds to 2} \cdot \text{(Glc)} \cdot \text{since fluxes are expressed in C3 units.}]

Flux through alanine aminotransferase is equal to aspartate accumulation: \([\text{Asp}]^{\text{Glu}} = [\text{OAA}^{\text{Glu}} \cdot (\text{OAA} \rightarrow \text{PEP}) \cdot (\text{PEP} \rightarrow \text{3PG} \cdot (\text{3PG} \rightarrow \text{Glc}) \cdot \text{corresponds to 2} \cdot \text{(Glc)} \cdot \text{since fluxes are expressed in C3 units.}]

Flux through alanine aminotransferase is equal to alanine accumulation: \([\text{Ala}]^{\text{Glu}} = [\text{OAA}^{\text{Glu}} \cdot (\text{OAA} \rightarrow \text{PEP}) \cdot (\text{PEP} \rightarrow \text{Pyr}) \cdot (\text{Pyr} \rightarrow \text{Ala}).]

Net flux through glutamate dehydrogenase is equal to the net flux of glutamate conversion into \(\alpha\)-ketoglutarate minus the net flux through transaminases.

Flux through \(\alpha\)-ketoglutarate dehydrogenase, \([\alphaKGdH]), is given by the amount of \(\alpha\)-ketoglutarate converted into succinyl-CoA and subsequently into oxaloacetate (see Equations 8, 9, 72, and 75).

\([\alphaKGdH] = X \cdot ([\text{Glu} \rightarrow \alphaKG] \cdot (\alphaKG \rightarrow \text{OAA}) + [\text{OAA}^{\text{Glu}} \cdot ([\text{OAA} \rightarrow \text{Cit}] + (\text{OAA} \rightarrow \text{PEP}) \cdot (\text{PEP} \rightarrow \text{Pyr}) \cdot (\text{Pyr} \rightarrow \text{AcCoA}) \cdot (\text{AcCoA} + \text{OAA} \rightarrow \text{Cit})] \cdot (\alphaKG

\rightarrow \text{OAA}) = X \cdot ([\text{Glu} \rightarrow \alphaKG] \cdot (\alphaKG \rightarrow \text{OAA}) + [\text{OAA}^{\text{Glu}} \cdot (\text{TCA}]) + (\text{AcCoA} + \text{OAA} \rightarrow \text{Cit}) \cdot (\text{AcCoA \ parallel OAA}) \cdot (\text{Eq. 81})]

Since oxaloacetate is produced either by pyruvate carboxylase or \(\alpha\)-ketoglutarate dehydrogenase, flux through pyruvate carboxylase is equal to \([\text{OAA}^{\text{Glu}} \cdot (\text{OAA}) \cdot (1 \rightarrow 2\cdot \text{OAA})].]

As shown in a previous study (1), flux of oxaloacetate equilibration with fumarate is equal to \([\text{OAA}^{\text{Glu}} \cdot 2\cdot \text{OAA} \cdot (1 \rightarrow 2\cdot \text{OAA})].]

DISCUSSION

Our model, which can be used at any time point, is based on proportions of metabolite conversion. It allows us to ignore the status of the system irrespective of whether or not it is in a steady state since the resynthesis of the substrate carbons on which most of the calculations are based is small. Other models (3–14) are based on kinetic reaction rates but were applied under steady state conditions.

With our model, we calculated mean fluxes related to glutamate metabolism over 1 h of incubation; for this we divided the amount per g dry wt of the metabolite of interest (that was formed during the incubation) by the incubation time (1 h in this study). Similarly, it should also be underlined that our parameter values were not necessarily constant with time but were also mean values. For example, it is clear that, at early times of incubation, the \(13^C\) atoms entering the glutamine pool were significantly diluted by the glutamine already present in the tubules at zero time. This resulted in a low proportion of glutamine converted into glutamate (\([\text{Gln} \rightarrow \text{Glu}]). However, since the glutamine present at zero time was only a small fraction (less than 10%) of the total glutamine found after 60 min of incubation, we may conclude that the impact of what happened during early times was limited when compared with what happened over a 60-min incubation period.

Most of the proportions and equations we used to calculate enzymatic fluxes were derived from the fate of the C-3 of glutamate, which provides more information about all the turns of the tricarboxylic acid cycle and the other cycles than that of the C-5 and the C-1 of glutamate; indeed the latter carbons are released as \(CO_2\) and recovered in the non-volatile products of glutamate metabolism that accumulate only before the end of the first turn of the tricarboxylic acid cycle. In the present study, the data obtained with unlabeled glutamate plus labeled \(CO_2\) as substrate were used to calculate the equilibrium of oxaloacetate with fumarate.

It should be emphasized that many proportions could also have been calculated by using different sets of data, yielding similar results. This illustrates the flexibility of our mathematical model which can also be applied not only to glutamate metabolism in tissues other than the kidney but also to data obtained with substrates other than glutamate and under many physiopathological conditions.

This model, which includes the simultaneous operation of five interdependent metabolic cycles, represents a significant progress when compared with our previous model of glucose metabolism which involved only three metabolic cycles (1). Indeed, in the present model, the glutamate resynthesized and further metabolized is taken into account. Moreover, this new model allows the calculation of the simultaneous synthesis and degradation of glutamate and \(\alpha\)-ketoglutarate. Note here that Shulman and co-workers (11, 12, 15) were also able to calculate the \(\alpha\)-ketoglutarate -- glutamate exchange in rat and human brain \textit{in vivo}. In addition, our model of glutamate metabolism allows us to calculate the simultaneous synthesis and degradation of glutamine that result from opposing unidirectional fluxes through glutamine synthetase and glutaminase. Such a more complex description of glutamate metabolism than previously described was made possible by the careful analysis of the labeling pattern and the amount of label recovered in glutamate and glutamine that accumulated after having passed through the tricarboxylic acid cycle.

It should be pointed out that in studies performed \textit{in vitro} like the present one, it is possible to obtain detailed NMR data, which in turn calls for a highly detailed analysis in order to obtain as much information as possible. Studies performed \textit{entirely in vivo}, in contrast, avoid physiological uncertainties associated with differences of metabolism \textit{in vivo} and \textit{in vitro} but yield much less detailed information due to reduced spectral resolution and limited averaging time.

Finally, depending on experimental data available, our model permits us to calculate either net or unidirectional enzymatic fluxes through the cycles involved in glutamate me-
tabolism and brings new insights into the complexity of such a metabolism in mammalian cells.

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