

Estimation of metabolic flux from dominant rate constants in vivo: application to brain and heart

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Abstract

In an earlier paper (Cohen and Bergman, *Am. J. Physiol.* 268 (1995) E397), we explored the relationship between the exponents in the exponential curve fit to isotopic enrichment versus time and the fractional turnover rate of the largest metabolic pool in the pathway. Here we present the analysis on a more rigorous footing and apply it to questions of cerebral and cardiac metabolism. Our emphasis in this paper is to describe and justify mathematically an approach for analysis of metabolic dynamics, not with the intention of replacing the use of numerical software for estimation of flux rates but for giving the scientist the opportunity to examine the system in an approximate manner, and thereby to check not only that the results of the numerical solution are the correct solutions to the equations but also that the equations portray the correct simplification of the metabolic pathway.

We introduce the “dominant rate constant” as a tool for deriving algebraic formulas relating rates of metabolic flux, sizes of metabolic pools, and the dynamics of isotopic enrichment. Illustrations of such algebraic formulas are provided for the rates of the citric acid cycle (CAC), glycolysis and glutamine synthesis in brain, as well as the rate of the CAC in heart. In addition, we prove that formulas for estimation of rates of glycolysis and of the CAC depend critically on the fractional turnover rates of lactate and glutamate, respectively.

The justification for analysis of simulated data is that we are studying the effects of simplifications of metabolic models on the accuracy of estimation of metabolic pathways. Our use of the dominant rate constant is an analytical convenience that allows us to assess proposed simplifications of metabolic pathways.

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1. Introduction

The purpose of computing is insight, not numbers—
(R.W. Hamming, 1973).

Owing to the sensitivity limitations of in vivo nuclear magnetic resonance (NMR) spectroscopy, the concentrations of NMR detectable atoms (e.g., ¹³C concentration at a given carbon position) must lie in the millimolar range (or higher) in order to be measured. For this reason, the concentration of isotopes having a

low natural abundance (such as ¹³C) often needs to be enriched by administering exogenously labeled compounds. In metabolic studies, isotopically labeled compounds (such as [1-¹³C]glucose) are typically administered and the isotopic enrichments of NMR visible metabolic pools (such as [3-¹³C]lactate) are measured over time. One is therefore compelled to infer rates of metabolic flux from observation of those pools whose concentrations and enrichments happen to be large enough to permit measurement by NMR spectroscopy.^{1,2} Estimates of metabolic flux are obtained from

Abbreviations: CAC-citric acid cycle; v_{CAC} -rate of the citric acid cycle; v_{TA} -rate of flux catalyzed by aspartate aminotransferase; v_{ANA} -rate of anaplerotic flux; γ -relative anaplerotic flux; v_{GS} -rate of flux catalyzed by glutamine synthetase; $L_{\text{C-2B}}$ -the magnitude of the subdominant rate constant of carbon C-2 of glutamate.

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¹Mass spectrometry, a more sensitive method for measuring isotopic enrichment, is limited by the need to extract a sample of the tissue whose metabolic intermediates are to be measured.

²Measurement of specific activity of compounds containing radioactive isotopes (such as ¹⁴C) does not provide measurement of either positional isotopomers (as measured by NMR spectroscopy) or mass

computer programs which minimize the error of the solution to a set of differential equations with respect to observed time courses of isotopic enrichment. These differential equations are often written to describe a simplified model of the metabolic system, omitting those pools and chemical reactions that are considered not to impact the estimation of parameters of interest.³ Given the veracity of these differential equations and making assumptions about the statistical distribution of errors, one can obtain estimates of flux rates as well their standard errors, thereby evaluating the precision of these estimates of flux.

For a complicated set of ordinary differential equations that describe the dynamics of isotopic labeling of metabolic species, one may feel helpless to question or to doubt the effect of model simplification on its applicability. By analysis of dominant rate constants for the glycolytic pathway and for the citric acid cycle (see Section 3), we demonstrate the limits of common models used to estimate the rates of these fluxes. It is our purpose to provide an analytical technique that allows one to estimate the value of one of the rate constants of the isotopic labeling of a given atom within a compound, and to use this rate constant in an algebraic formula for the purpose of estimating the absolute rate of metabolic flux of a given pathway.

We have investigated questions related to the *validity* of models used to estimate rates of flux, i.e., whether the solution of the differential equations for a given simplified model would yield an accurate estimate of a given flux rate. We propose several simple analytical techniques with which to derive the fundamental (“dominant”) rate constants of isotopic enrichment of selected intermediate pools, during administration of an isotopically labeled compound. Comparison of these estimates of rate constants with experimentally determined rate constants provides a test of the validity of a simplified model. Analysis of simplified models of metabolic pathways allows one to determine if sufficient measurements can be made to enable a given flux to be estimated, and under which physiological conditions a given method may no longer provide accurate estimates. We demonstrate that estimates of rates of metabolic flux can be obtained directly from the experimentally determined rate constants of isotopically labeled com-

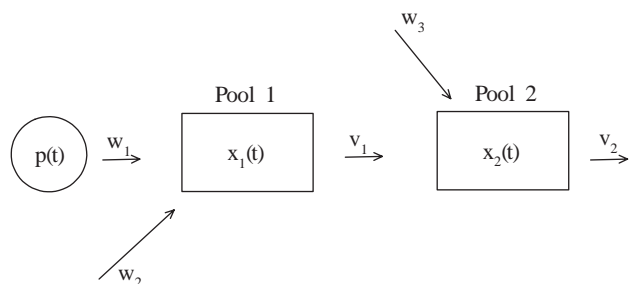


Fig. 1. Model of a metabolic pathway, in which pool 1 is the unique source of isotopic enrichment into pool 2. The fractional enrichments of pools 1 and 2 are $x_1(t)$ and $x_2(t)$, respectively. Flux rates (mol/min) are denoted w_1 , w_2 , w_3 (from pools other than pool 1 and pool 2) and v_1 , v_2 . The flux labeled w_1 has fractional isotopic enrichment $p(t)$; fluxes labeled w_2 and w_3 are have zero enrichment. The *fractional turnover rate* of pool 2 equals v_2/M_2 (min^{-1}), where v_2 (mol/min) equals the rate of disposal of pool 2 (i.e., rate of conversion of molecules of pool 2 to all other pools) and M_2 is the number of moles in pool 2. Note that whereas fractional turnover rate is a non-negative quantity, a *rate constant* may be (and usually is) a negative number.

pounds, using algebraic formulas instead of differential equations.⁴

2. Methods

Next we discuss the use of differential equations and dominant rate constants for analysis of the time course of isotopic enrichment.

2.1. Differential equations for isotopic enrichment

Consider the fragment of a metabolic pathway shown in Fig. 1 and suppose that pool 1 is the sole immediate precursor of pool 2 that contains any⁵ isotopic label (e.g., ^{13}C). We write the equations for conservation of mass of pool 2:

$$dM_2(t)/dt = v_1(t) + w_3(t) - v_2(t) \quad (1)$$

(where $M_2(t)$ equals the number of moles of molecules in pool 2) and for the conservation of mass of ^{13}C atoms

⁴Abstracts of preliminary versions of this material have been published: Cohen and Bergman. Estimation of the rate of the citric acid cycle in heart without the solution of differential equations. In *Proceedings of the Second Meeting of the Society of Magnetic Resonance*, 1994, Vol. 3, p. 1235; Cohen and Bergman. Estimation of the rate of the tricarboxylic acid (TCA) cycle using [^{13}C]glucose without the solution of differential equations. In *Proceedings of the Society of Magnetic Resonance*, 1995, Vol. 1, p. 303; Cohen and Bergman, 1996. Improved estimation of the rate of flux catalyzed by glutamine synthetase in vivo, using [^{15}N]-NMR spectroscopy. *J. Neurochem.*, 66, (Suppl. 1) S-70.

⁵To illustrate the mathematical analysis, we shall assume zero natural abundance of ^{13}C .

(footnote continued)

isotopomers (as measured by mass spectrometry). However, chemical separation and degradation steps can subsequently be performed in order to determine the fractional enrichment of an individual carbon.

³Alternatively, a comprehensive set of differential equations are written and those pools and chemical reactions which contribute little to the estimate of the flux rates of interest are determined by computer simulation, using an exhaustive search of the parameter space (trial and error).

within pool 2:

$$dM_2^*(t)/dt = x_1(t)v_1(t) - x_2(t)v_2(t) \quad (2)$$

where $M_2^*(t)$ equals the moles of isotopically labeled molecules in pool 2, and $x_1(t)$ (or $x_2(t)$) is the fractional enrichment of pool 1 (or pool 2, respectively) at time t :

$$x_1(t) = M_1^*(t)/M_1(t), \quad (3)$$

$$x_2(t) = M_2^*(t)/M_2(t) \quad (4)$$

and where $M_1(t)$ equals the number of moles in pool 1. Under the assumption of metabolic steady state ($dM_1/dt = dM_2/dt = dw_1/dt = dw_2/dt = dw_3/dt = dv_1/dt = dv_2/dt = 0$), Eq. (2) can be written as follows:

$$M_2 dx_2/dt = x_1(t)v_1 - x_2(t)v_2 \quad (5)$$

and the following relationships can be shown:

$$v_1 = w_1 + w_2, \quad (6)$$

$$v_2 = v_1 + w_3. \quad (7)$$

By definition, at isotopic steady state, $dM_1^*(t)/dt = dM_2^*(t)/dt = 0$, resulting in the following relationship of v_1 , v_2 and the steady state enrichments (x_1^{ss} , x_2^{ss}) of pools 1 and 2 (obtained from Eq. (5)):

$$v_1 = v_2 x_2^{ss} / x_1^{ss}. \quad (8)$$

Taking the Laplace transform⁶ of both sides of Eq. (5) yields

$$\bar{x}_2 = (\bar{x}_1 v_1 + M_2 x_2(0)) / (M_2(s + v_2/M_2)). \quad (9)$$

For purposes of illustration,⁷ we assume that $x_1(t) = p$ is constant (so that $\bar{x}_1 = p/s$) and rewrite Eq. (9), using the method of “expansion in partial fractions” (Arfken and Weber, 1995; Hildebrand, 1962; Oppenheim and Willsky, 1983):

$$\bar{x}_2 = (pv_1/v_2)(1/s - 1/(s + v_2/M_2)) + x_2(0)/(s + v_2/M_2). \quad (10)$$

Next (taking the inverse Laplace transform) we obtain a formula for the fractional enrichment $x_2(t)$ of pool 2 at time t :

$$x_2(t) = (v_1/v_2)p(1 - e^{-v_2 t/M_2}) + x_2(0)e^{-v_2 t/M_2} \quad (11)$$

illustrating that enrichment of pool 2 is a monoexponential function of time, having rate constant λ equal to $-v_2/M_2$, (where v_2 is the turnover of pool 2). In general, the flux v_1 from pool 1 to pool 2 can be estimated from λ and M_2 , using Eq. (8).

⁶For any function $f(t)$, we let $\mathbb{L}\{f\}$, $\bar{f}(s)$, or \bar{f} denote its Laplace transform (see Appendix A).

⁷More generally, for an arbitrary function $x_1(t)$, the following formula can be derived from Eq. (9) using the convolution theorem (Arfken and Weber, 1995; Hildebrand, 1962)

$$x_2(t) = (v_1/M_2) \int_0^t x_1(u) e^{-v_2(t-u)/M_2} du + x_2(0) e^{-v_2 t/M_2}.$$

2.2. Dominant rate constants

As we have illustrated (Eq. (11)), the solution to the differential equations representing the fractional enrichment x_i of a particular carbon of an individual metabolite is the sum of exponential terms (assuming metabolic steady state):

$$x_i(t) = a_0 + a_1 e^{\lambda_1 t} + a_2 e^{\lambda_2 t} + a_3 e^{\lambda_3 t} + \dots + a_n e^{\lambda_n t} \quad (12)$$

where we may assume⁸ the terms have been ordered so that $0 > \lambda_1 > \lambda_2 \geq \lambda_3 \geq \dots \geq \lambda_n$. The dominant rate constant of $x_i(t)$ is defined to be λ_1 , which represents the exponential term with the slowest rate of decay over time: $1 > e^{\lambda_1 t} > e^{\lambda_2 t} \geq e^{\lambda_3 t} \geq \dots \geq e^{\lambda_n t} > 0$, for all $t > 0$ (Fig. 2). The subdominant rate constant is λ_2 .

The significance of the dominant rate constant λ_1 of $x_i(t)$ is that, after some period of time, the dynamics of $x_i(t)$ are entirely determined by λ_1 . Mathematically, for each increase of time by $1/(\lambda_1 - \lambda_2)$ units, the value of $e^{\lambda_2 t}/e^{\lambda_1 t}$ decreases to 36.8% of its former value. At $t = 3/(\lambda_1 - \lambda_2)$, $t = 4/(\lambda_1 - \lambda_2)$, and $t = 5/(\lambda_1 - \lambda_2)$ units of time, for example, the ratio $e^{\lambda_2 t}/e^{\lambda_1 t}$ has decreased from 100% to 4.98%, 1.83%, and 0.67%, respectively. If the rate constants λ_1 and λ_2 are well separated, i.e., $\lambda_1 \gg \lambda_2$, then the decrease in the ratio $e^{\lambda_2 t}/e^{\lambda_1 t}$ is precipitous; however, no matter how close the dominant and subdominant rate constants may be, eventually the dynamics of $x_i(t)$ will be entirely determined by $e^{\lambda_1 t}$.

The coefficients $\{a_j, j \geq 0\}$ of $x_i(t)$ may be obtained by taking the Laplace transform (see appendix) of $x_i(t)$ in Eq. (12):

$$\bar{x}_i(s) = a_0/s + a_1/(s - \lambda_1) + a_2/(s - \lambda_2) + a_3/(s - \lambda_3) + \dots + a_n/(s - \lambda_n) \quad (13)$$

and observing that

$$a_0 = \lim_{s \rightarrow 0} \{s \bar{x}_i(s)\} \text{ and} \\ a_j = \lim_{s \rightarrow \lambda_j} \{(s - \lambda_j) \bar{x}_i(s)\}, \text{ for all } j > 0 \quad (14, 15)$$

(cf refs. Arfken and Weber, 1995; Hildebrand, 1962; Oppenheim and Willsky, 1983). Therefore, from $\bar{x}_i(s)$, and taking the appropriate limits, one can obtain the coefficients of the exponential terms of $x_i(t)$.

⁸A metabolic pathway is “separable” if it can be described in terms of smaller pathways (numbered 1, 2, ..., K) such that pathway i does not receive flux from pathway j for $j > i$ (Jacquez, 1985). (Alternatively, a pathway is separable iff its digraph is not strongly connected.) It can be demonstrated (Hearon, 1963) that the dominant rate constant of a non-separable metabolic pathway is a simple (i.e., non-repeated) real eigenvalue, so that $\lambda_1 \neq \lambda_2$. Conservation of mass implies that any complex eigenvalues have negative real parts. Entry of unlabeled molecules into the system ensures that the matrix describing the differential equations is diagonally dominant and hence nonsingular (Hearon, 1963). Additionally, the fluctuation and imprecision of measurement of flux rates and pool sizes allows for the assumption of distinct solutions for the characteristic polynomial, yielding non-repeated eigenvalues.

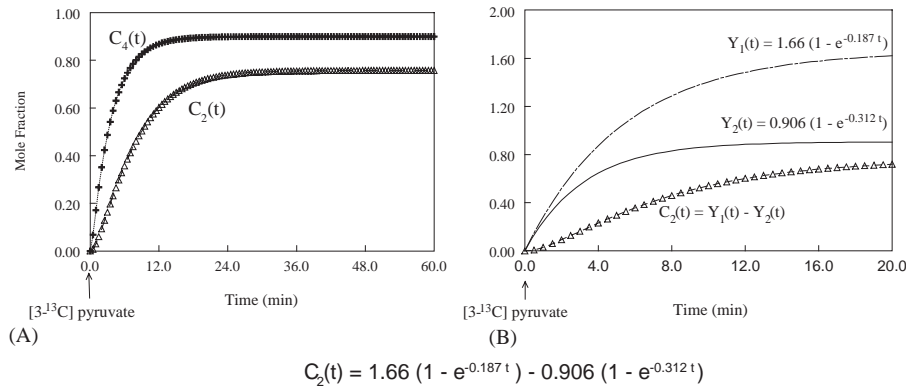


Fig. 2. Comparison of the fractional turnover rate and the dominant rate constant for the fractional enrichment $C_2(t)$ of carbon C-2 of glutamate. (A) Simulation of labeling of C-2 and C-4 of glutamate by the citric acid cycle (Fig. 3) during provision of $[3-^{13}\text{C}]$ pyruvate. Data are given in Table 1, with the following values for three flux rates (in units of $\mu\text{mol/g-dry wt/min}$): v_{CAC} (8.28), v_{TA} (22.9), v_{ANA} (2.484). (B) Contribution of the individual exponential terms to the net value of $C_2(t)$ during the first 20 min. The dominant and subdominant rate constants of $C_2(t)$ are -0.187 min^{-1} and -0.312 min^{-1} , respectively. For comparison, the fractional turnover rate of glutamate in the simulation is 1.12 min^{-1} .

2.3. Software packages

Estimation of parameters (least squares' estimation) using the Marquardt-Levenberg curve-fitting algorithm was performed using MLAB (Civilized Software, Inc.) or SlideWrite Plus (Advanced Graphics Software, Inc.). Symbolic mathematics was performed with Mathematica (Wolfram Research, Inc.).

3. Results

We now derive formulas for the rates of the citric acid cycle, of glycolysis and of the reaction catalyzed by glutamine synthetase by application of the concept of a dominant rate constant, making use of a computer simulation and mathematical analysis. We investigate aspects of the next slower (i.e., subdominant) rate constant of glutamate and then present mathematical properties of dominant rate constants, including their quantitative relationship to fractional turnover rates, and coefficients of exponential terms (describing the enrichment of a metabolic pool).

3.1. Estimation of fluxes using the dominant rate constant

Next we explain how to use dominant rate constants as an aid to thinking about estimates of metabolic flux rates. Given experimental measurements of isotopic enrichment of metabolites during administration of labeled substances (such as presented in Fig. 2A), the solution to the differential equations describing the transfer of carbon atoms within the metabolic pathway may yield a solution which (in theory) is the sum of many exponential terms. In practice, however, owing to noise in the biological system and random error in the

measurements, it is not possible to identify more than one or possibly two exponential terms.

Our approach is to deal with the empirical rate constants and to obtain an algebraic formula for a given rate of flux in terms of the dominant (slowest) rate constant, as well as the mass of a small number of metabolic pools. We fit the data to a sum of one or two exponential terms, thereby obtaining the empirical rate constants (see Fig. 2B). Then we derive an equation for the rate of flux of a given reaction, by analyzing the differential equations in the Laplace domain (see above).

3.1.1. Citric acid cycle in heart: labeled anaplerosis

Using the notion of dominant rate constant, we have previously derived a formula for the rate v_{CAC} of the citric acid cycle in heart during administration of a constant supply of $[2-^{13}\text{C}]$ acetyl CoA (Cohen and Bergman, 1995). We showed how v_{CAC} can be estimated from the observed (empirical) rate constants of C-2 and C-4 of glutamate (cf Fig. 2). Presently, we have extended the analysis to include administration of $[3-^{13}\text{C}]$ pyruvate or $[1-^{13}\text{C}]$ glucose and allowing labeled anaplerosis from pyruvate, i.e., conversion of $[^{13}\text{C}]$ pyruvate to $[^{13}\text{C}]$ oxaloacetate or $[^{13}\text{C}]$ malate by reactions catalyzed by pyruvate carboxylase or malic enzyme, respectively (Table 1).

The algebraic formulas for the estimates of the magnitudes of the dominant rate constants $L_{\text{C-2}}$, $L_{\text{C-3}}$, and $L_{\text{C-4}}$ of glutamate carbons C-2, C-3 and C-4, respectively, are the following (Fig. 3):⁹

⁹As in Cohen and Bergman (1995), the following assumptions need to be satisfied: $[\text{cit}]/[\text{glu}] < 1 + v_{\text{CAC}}/v_{\text{TA}}$ and $(M - [\text{glu}])/M < 1/(1 + 2\gamma)$.

Table 1

Ratio of estimated to actual values of v_{CAC} , v_{TA} , L_{C-2} , L_{C-3} , and L_{C-4} for different pre-set (i.e., “actual”) rates of y , v_{CAC} and v_{TA} (Fig. 3) in simulations for which [3-¹³C]pyruvate was administered at a constant rate at time $t = 0$ min

Actual value					Estimated value/Actual value				
v_{CAC}	v_{TA}	L_{C-2}	L_{C-3}	L_{C-4}	v_{CAC}	v_{TA}	L_{C-2}	L_{C-3}	L_{C-4}
<i>Relative anaplerosis: $y = 0$</i>									
4.14	9.16	0.1763	0.1763	0.3437	1.0657	0.9288	0.9597	0.9597	0.9809
8.28	9.16	0.3065	0.3065	0.5074	1.1395	0.8526	0.9565	0.9565	1.0164
16.56	9.16	0.4972	0.4972	0.6971	1.2761	0.8832	0.9306	0.9306	1.0066
4.14	22.90	0.0879	0.0879	0.1631	1.1928	0.4869	0.9288	0.9288	1.0233
8.28	22.90	0.1562	0.1562	0.2891	1.0641	0.8499	0.9725	0.9725	1.0023
16.56	22.90	0.2842	0.2842	0.4637	1.1493	0.8668	0.9391	0.9391	0.9886
4.14	32.06	0.0628	0.0628	0.1231	1.0878	0.5690	0.9656	0.9656	1.0147
16.56	32.06	0.2336	0.2336	0.3738	1.3200	0.6846	0.8910	0.8910	0.9962
8.28	32.06	0.1197	0.1197	0.2231	1.0948	0.7335	0.9605	0.9605	1.0053
<i>Relative anaplerosis: $y = 0.1$</i>									
4.14	9.16	0.1918	0.1919	0.3445	1.0615	0.9428	0.9620	0.9614	0.9785
8.28	9.16	0.3372	0.3375	0.5241	1.1599	0.8918	0.9368	0.9360	0.9840
16.56	9.16	0.5034	0.5034	0.6884	1.1492	0.9063	0.9745	0.9745	1.0193
4.14	22.90	0.0901	0.0902	0.1617	1.0630	0.6483	0.9897	0.9891	1.0323
8.28	22.90	0.1688	0.1688	0.2839	1.0859	0.7699	0.9778	0.9774	1.0207
16.56	22.90	0.3010	0.3011	0.4500	1.1864	0.7913	0.9530	0.9526	1.0188
4.14	32.06	0.0674	0.0674	0.1231	1.0511	0.6653	0.9847	0.9845	1.0142
16.56	32.06	0.2412	0.2414	0.3653	1.2531	0.6896	0.9316	0.9309	1.0193
8.28	32.06	0.1322	0.1323	0.2211	1.1616	0.6208	0.9465	0.9458	1.0142
<i>Relative anaplerosis: $y = 0.3$</i>									
4.14	9.16	0.2123	0.2128	0.3391	1.0453	0.9271	0.9798	0.9777	0.9941
8.28	9.16	0.3666	0.3673	0.5115	1.1910	0.8342	0.9541	0.9524	1.0084
16.56	9.16	0.5328	0.5324	0.6771	1.1499	0.8847	0.9957	0.9963	1.0364
4.14	22.90	0.1005	0.1008	0.1601	1.0576	0.6310	1.0043	1.0014	1.0428
8.28	22.90	0.1873	0.1876	0.2789	1.1163	0.6952	0.9873	0.9855	1.0391
16.56	22.90	0.3278	0.3283	0.4391	1.2655	0.7153	0.9655	0.9641	1.0442
4.14	32.06	0.0759	0.0760	0.1218	1.0669	0.5856	0.9901	0.9885	1.0250
8.28	32.06	0.1476	0.1481	0.2173	1.2192	0.5388	0.9530	0.9498	1.0319
16.56	32.06	0.2431	0.2435	0.3521	1.0398	0.8035	1.0260	1.0241	1.0577

For each set of values assigned to y , v_{CAC} and v_{TA} , simulated data (the fractional enrichments of carbons of glutamate at successive time points) were obtained by solving the differential equations numerically (see Appendix A of Cohen and Bergman, 1997). As in Cohen and Bergman (1995), changes in v_{TA} did not alter the mass action ratio of aspartate aminotransferase, because the concentrations of glutamate and of aspartate were multiplied by the same number, preserving the value of $v_{TA}/[\text{glutamate}]$ at 1.12 min^{-1} . The rate of flux (v_{PDH}) catalyzed by pyruvate dehydrogenase complex equals $80.0 \mu\text{mol/g-dry wt-min}$; the rate of the reverse flux of fumarase equals 10 times v_{CAC} ; the rate of anaplerosis (v_{ANA}) equals yv_{CAC} ; and the rate of influx of acetyl CoA equals v_{CAC} . Actual values of L_{C-2} , L_{C-3} , and L_{C-4} are in units of min^{-1} and were obtained by fitting a single exponential function (for L_{C-4}) or a sum of two exponential terms (for L_{C-2} and L_{C-3}) to the simulated data. v_{CAC} and v_{TA} are in units of $\mu\text{mol/g dry wt-min}$. Estimated values of v_{CAC} , v_{TA} , L_{C-2} , L_{C-3} , and L_{C-4} were obtained by use of Eqs. (16)–(20). The fractional turnover rate of pyruvate is $(v_{ANA} + v_{PDH})/[\text{pyruvate}]$, and $[\text{pyruvate}] = 0.2 \mu\text{mol/g dry wt}$.

$$L_{C-2} = \frac{v_{CAC}v_{TA}}{[\text{glutamate}](v_{CAC} + v_{TA}) + [\alpha\text{-ketoglutarate}]v_{TA} + (Mv_{TA}/(1 + 2y))}, \quad (16)$$

$$L_{C-3} = L_{C-2}, \quad (17)$$

$$L_{C-4} = \frac{v_{CAC}v_{TA}}{[\text{glutamate}](v_{CAC} + v_{TA}) + [\alpha\text{-ketoglutarate}]v_{TA}}, \quad (18)$$

where y equals anaplerotic flux divided by citric acid cycle flux (v_{CAC}), M is the sum of the concentrations of glutamate, aspartate, and the intermediates of the citric acid cycle (excluding AcCoA). Although labeled

anaplerosis affects the steady state fractional enrichment of glutamate (Cohen and Bergman, 1997), the formulas for the estimates of the dominant rate constants of carbons C-2, C-3 and C-4 of glutamate are the same as for the case of unlabeled anaplerosis and no reverse flux of fumarase (i.e., flux “e” = 0 in Fig. 3).¹⁰ We have verified this prediction by numerically solving the

¹⁰In Cohen and Bergman (1995), the formulas were given in terms of the parameter β , where $\beta = 1/(1 + y)$.

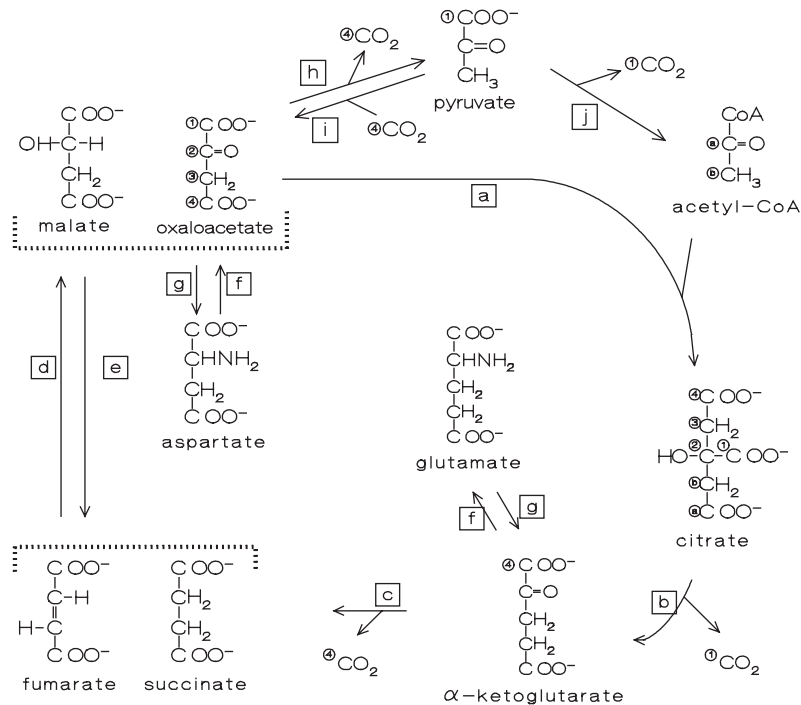


Fig. 3. Schematic of the citric acid cycle and associated reactions in myocytes, showing transfer of carbons. Chemical fluxes are denoted by letters: (a) citrate synthase; (b) aconitase and isocitrate dehydrogenase; (c), α -ketoglutarate dehydrogenase complex and succinyl CoA synthetase; (d) fumarase; (e) fumarase (reverse); (f) aspartate aminotransferase; (g) aspartate aminotransferase (reverse); (h) malic enzyme and pyruvate carboxylase (cataplerosis); (i) malic enzyme and pyruvate carboxylase (reverse, anaplerosis); and (j) pyruvate dehydrogenase complex. Fumarate and succinate are combined into one pool, as are malate and oxaloacetate. Reversible fluxes (catalyzed by fumarase, aspartate aminotransferase, malic enzyme, and pyruvate carboxylase) have been separated into “forward” and “reverse” fluxes.

equations for fractional enrichment of carbons in the model of the citric acid cycle presented in Fig. 3, for values of reverse flux of fumarase between zero and 10 v_{CAC} (data not shown). Interestingly, the formulas for $L_{\text{C-2}}$ and $L_{\text{C-3}}$ are equal, even if coefficients of dominant exponential terms for the fractional enrichments of carbons C-2 and C-3 of glutamate are different.

Solving Eqs. (16)–(18) yields the following equations:

$$v_{\text{CAC}} = \frac{ML_{\text{C-2}}L_{\text{C-4}}}{(1 + 2y)(L_{\text{C-4}} - L_{\text{C-2}})}, \quad (19)$$

$$v_{\text{TA}} = \frac{v_{\text{CAC}}L_{\text{C-4}}[\text{glutamate}]}{v_{\text{CAC}} - L_{\text{C-4}}([\text{glutamate}] + [\alpha\text{-ketoglutarate}])}. \quad (20)$$

We tested the accuracy of these equations (Table 1) during changes in the fractional enrichment of carbons of pyruvate for three different values of v_{CAC} , v_{TA} and relative anaplerosis (y). The accuracy of Eqs. (16)–(18) is excellent (based on the performance on these 27 cases): all but one estimate of $L_{\text{C-2}}$ and $L_{\text{C-3}}$, and every estimate of $L_{\text{C-4}}$ is within 10% of the correct value. Estimates of v_{CAC} were within 10% (20%, 30%) of the actual value in 12 (22, 26) out of 27 cases (respectively). Estimation of v_{TA} was not nearly as accurate—estimates were within 10% (20%, 30%) of the actual value in 4 (12, 16) out of

27 cases (respectively). Recalling that the formulas (Eqs. (19) and (20)) for v_{CAC} and v_{TA} were derived from those of $L_{\text{C-2}}$, $L_{\text{C-3}}$, and $L_{\text{C-4}}$ (Eqs. (16)–(18)), we conclude that numerical instability (sensitivity to parameter values) explains the relatively poor performance of Eq. (20). In fact, we have derived formulas for the sensitivity of these estimates (Cohen and Bergman, 1995), and shown that the sensitivity of the estimate of v_{TA} increases with the ratio $v_{\text{TA}}/v_{\text{CAC}}$, which is confirmed by Table 1.

We simulated the effects of different values of the fractional turnover rate F_{pyr} of pyruvate on the magnitude $L_{\text{C-4}}$ of the dominant rate constant of C-4 of glutamate, during administration of $[3\text{-}^{13}\text{C}]\text{pyruvate}$ (Fig. 4). Decreasing the fractional turnover rate of this precursor pool had marked effects on the value of $L_{\text{C-4}}$, showing an apparent linear relation in the semilog plot for a certain range of values of F_{pyr} . In fact, for fractional turnover rates of pyruvate below a certain threshold (determined by the masses of the pools of the citric acid cycle), the derivative of $L_{\text{C-4}}$ with respect to F_{pyr} equals k/F_{pyr} , for some constant k . Thus the value of the rate constant for C-4 of glutamate is extraordinarily sensitive to changes in the fractional turnover rate of pyruvate, whenever that rate is sufficiently small. Similarly with regard to transmission of rate constants

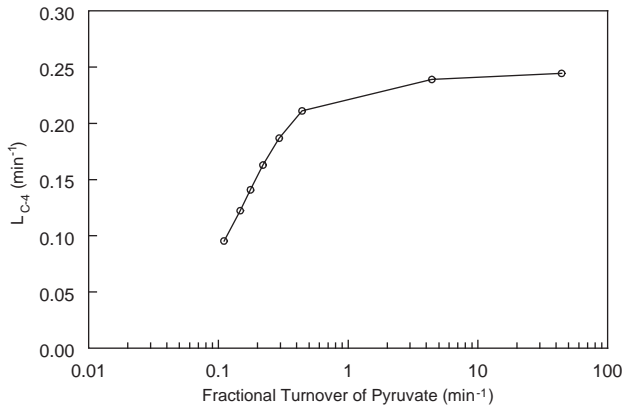


Fig. 4. Dependence of the magnitude (L_{C-4}) of the dominant rate constant of carbon C-4 of glutamate on the fractional turnover rate of pyruvate, during simulated administration of $[3-^{13}\text{C}]$ pyruvate. The differential equations solved using MLAB and assumptions of the model are given in Cohen and Bergman (1997). Values of the fractional enrichment of C-4 versus time were fit with a single exponential (Eq. (25)). Rates of flux (in $\mu\text{mol/g dry wt}\cdot\text{min}$): v_{CAC} (8.28), v_{TA} (22.9), v_{ANA} (0.828), v_{PDH} (8.0), influx of AcCoA (0.56), efflux of AcCoA (0.28). To vary the fractional turnover rate of pyruvate, the concentration of pyruvate was changed (and the rates of flux were unchanged), using the formula $(v_{\text{ANA}} + v_{\text{PDH}})/[\text{pyr}]$.

(Section 3.1.2), if the concentration of lactate decreases sufficiently, the time course of labeling of lactate will reflect the turnover of glucose (not lactate).

The formulas for the rate constants (Eqs. (16)–(18)) that are independent of the dynamics of pyruvate enrichment are the same as for the case of enrichment of acetyl CoA alone, reflecting the intrinsic dynamics of the citric acid cycle. As long as the fractional turnover rate of pyruvate is greater than these rate constants, the formulas estimating L_{C-2} , L_{C-3} and L_{C-4} are valid. However, when the fractional turnover rate of pyruvate is less than L_{C-2} (L_{C-3}), then these formulas are inadequate (Fig. 4).

3.1.2. Glycolysis in brain

To obtain a formula for estimation of the conversion of glucose to pyruvate in brain, we use a simplified model (Figs. 5 and 6 and Eq. (A.78) in the appendix). Letting $p_1(t)$ represent the fractional enrichment of C-1 of glucose, $x_1(t)$ represent the fractional enrichment of C-3 of pyruvate, $x_2(t)$ represent the fractional enrichment of C-3 of lactate, and $v_3 = 0$, we can obtain a formula for the rate $v_{\text{glycolysis}}$ (flux v_0 in Fig. 6) of glycolysis:

$$v_{\text{glycolysis}} \approx -L_{\text{dom}}\{x_2(t)\}\{[\text{lactate}] + [\text{pyruvate}]\}, \quad (21)$$

where $L_{\text{dom}}\{\cdot\}$ is the operation of obtaining the dominant rate constant of a function. There are two assumptions: (1) the flux v_1 catalyzed by lactate dehydrogenase is much larger than the difference between glycolytic flux and lactate efflux (v_2), i.e.,

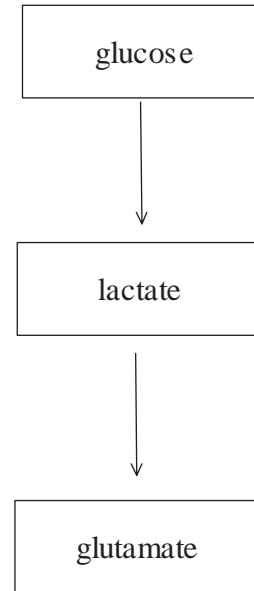


Fig. 5. Three pool model of glycolysis and citric acid cycle.

$v_1 \gg v_0 - v_2$; and (2) the fractional turnover rate of glucose C-1 exceeds $v_{\text{glycolysis}}/\{[\text{lactate}] + [\text{pyruvate}]\}$.¹¹

3.1.3. Glutamine synthesis in brain

The restriction of glutamine synthetase to glial cells makes that enzyme particularly attractive for analysis using the dominant rate constant. Existing techniques for measurement of the flux v_{GS} catalyzed by glutamine synthetase in rats in vivo using $[^{15}\text{N}]\text{NH}_3$ either (1) require knowledge of the enrichment of the precursor pool of ammonia (Kanamori and Ross, 1993); or rely on estimates of the initial rates of enrichment of glutamine (Kanamori et al., 1996). Use of the dominant rate constant to estimate v_{GS} , on the other hand, requires (1) measurement of the fractional enrichment of glutamine and (2) sufficient time to measure the rate constant λ_1 of isotopic enrichment. The resulting formula:

$$v_{\text{GS}} \cong -\lambda_1[\text{glutamine}] \quad (22)$$

may be obtained from a simple equation relating the change in isotopic enrichment of ammonia to the enrichment of the amide nitrogen in glutamine (Eq. (11)). Eq. (22) may be preferable to other methods for modeling of kinetics, because of the uncertainty of the fractional enrichment of the precursor pool and because of the predictable error incurred in the estimation of initial rate from the chord of a graph (rather than the tangent).¹²

¹¹Our theory predicts that if the latter assumption is not satisfied, then $-L_{\text{dom}}\{x_2(t)\}$ will equal the fractional turnover rate of glucose instead of the fractional turnover rate of the combined pools of pyruvate and lactate.

¹²Suppose v_{GS} is estimated from the graph of the enrichment of the amide nitrogen of glutamine versus time, by fitting a straight line

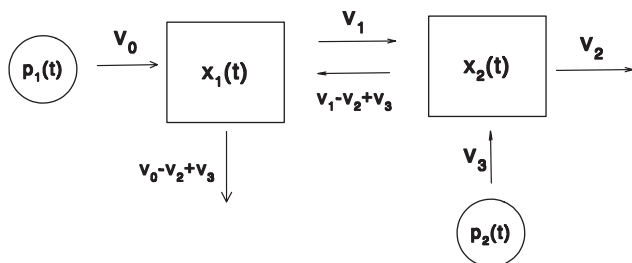


Fig. 6. General model of two exchanging pools (e.g., α -ketoglutarate and glutamate). Flux rates (v_0, v_1, v_2, v_3) and pool masses are constant (at metabolic steady state); $p_i(t)$ is the fractional enrichment of the immediate precursor of pool i at time t , for $i = 1, 2$; $x_i(t)$ is the fractional enrichment of pool i at time t , for $i = 1, 2$.

3.2. Investigation of the subdominant rate constant

3.2.1. Citric acid cycle in heart

From a practical as well as a theoretical viewpoint, the subdominant rate constant of C-2 glutamate is problematical. Simulating the metabolism of [2-¹³C]acetate in the heart (data presented in Cohen and Bergman, 1997), the fractional enrichment of C-2 of glutamate versus time was fitted with a sum of two exponential terms (although, as mentioned above, theoretically it represented the sum of a greater number of exponential terms). The magnitudes (L_{C-2} and L_{C-2B} , respectively) of the dominant and subdominant rate constants for this empirical curve approximation were obtained for each of 27 different cases representing different values of v_{CAC} , v_{CAC} , and anaplerotic flux (data not shown). On theoretical grounds, we predicted that L_{C-2B} would equal L_{C-4} (the magnitude of the dominant rate constant of C-4 of glutamate) under these conditions (Cohen and Bergman, 1995). However, numerical analysis of simulated data showed pronounced differences between L_{C-2B} and L_{C-4} , for many of the 27 cases in which parameters of a model of the CAC were varied.

Using Mathematica, we solved for the eigenvalues (rate constants) of the matrix describing the set of differential equations of the metabolic pathway we simulated. The matrix was different for each of the 27 cases examined (it depends on the parameter values), but in all 27 cases, the matrix's dominant eigenvalue of the enrichment of carbon C-2 of glutamate was equal to L_{C-2} and the subdominant eigenvalue (L_{C-2B}) was equal

to L_{C-4} (data not shown). This was in agreement with our theoretical predictions, but we could not explain what the value of L_{C-2B} meant. We determined that the lack of agreement between L_{C-2B} and L_{C-4} was not related to the stochasticity of the computer simulation and was related to the approximation by a sum of two exponentials of a sum of a considerable number of exponential terms (data not shown). Indeed, by equating the behavior of 2 exponential terms to a single one during early time points, we can derive the following approximation for L_{C-2B} :

$$L_{C-2B} \approx -(a_2\lambda_2 + a_3\lambda_3)/(a_2 + a_3), \quad (23)$$

where $a_2(a_3)$ and $\lambda_2(\lambda_3)$ are the coefficient and rate constant, respectively, for the second (third) term in the sum of exponential terms of Eq. (12) that describe the fractional enrichment of C-2 of glutamate versus time during a constant infusion of [2-¹³C]acetate.¹³ Agreement with L_{C-2B} was within 10% in 11 out of 27 cases and within 25% in 22 out of 27 cases (see Table 2). Intuitively, the subdominant rate constants are sandwiched between other rate constants (except for the most rapid rate constants) and are harder to estimate accurately than the dominant rate constant.

3.3. Properties of dominant rate constants

Each atom of each metabolite in a metabolic pathway has a dominant rate constant, which depends upon the flux rates of branches of the pathway and upon the size (mass) of each metabolic pool in the pathway. To enable dominant rate constants to serve as an aid to reasoning about isotopic dynamics of atoms, we have proposed and proven several of their properties.

3.3.1. The magnitude of the dominant rate constant of a catenary series of pools equals the smallest fractional turnover rate of the pools

The dynamics of isotopic labeling in a pool within a series of pools depends on the fractional turnover rate of each of the preceding pools (Fig. 7). Provided the magnitude of the dominant rate constant of the fractional enrichment $p(t)$ of the precursor pool is greater than each of the fractional turnover rates of the pools in the catenary system, the relationship of flux v to the magnitude $|\lambda_{\text{dom}}|$ of the dominant rate constant of a given pool (j) is

$$v = |\lambda_{\text{dom}}| M_i, \quad (24)$$

where M_i is the mass (number of moles) in pool i ($i \leq j$) for which the fractional turnover rate equals the magnitude of the dominant rate constant.

(footnote continued)

through the first 3 or 4 time points (and assuming a constant fractional enrichment and constant concentration of ammonia). Let R be the ratio of v_{GS} obtained in this manner (v_{GS} = slope (at time $t = 0$) divided by fractional enrichment of ammonia) to the actual value of v_{GS} . Then one can prove that R is independent of time but depends on the rate constant λ (obtained from Eq. (12), with $n = 1$) as well as on the time period Δt during which the graph is assumed to be linear: $R = (e^{\lambda\Delta t} - 1)/\lambda\Delta t$. Equivalently, $R = -\alpha/(\ln(1 - \alpha))$, where $\alpha = x_2(\Delta t)/x_2(\infty)$; for $\alpha = 5/8$, as in Kanamori et al. (1996), $R = 0.64$.

¹³The formula can be derived by matching the initial slopes of the $q(t)$ and $r(t)$, as defined in the following: $q(t) = a_2(1 - e^{\lambda_2 t}) + a_3(1 - e^{\lambda_3 t})$; $r(t) = (a_2 + a_3)(1 - e^{-L_{C-2B} t})$.

Table 2

Value of the magnitude L_{C-2B} of the subdominant rate constant of C-2 of glutamate for 27 cases in which the effects of administering $[2-^{13}C]$ acetate were simulated, excluding the possibility of recycling of label via anaplerosis

Actual value					Estimated value/Actual value		
v_{CAC}	v_{TA}	L_{C-2}	L_{C-4}	L_{C-2B}	L_{C-2}	L_{C-4}	L_{C-2B}
<i>Relative Anaplerosis: y = 0</i>							
4.1814	9.4485	0.1664	0.3510	0.3561	1.0379	0.9770	1.4925
7.8246	8.6862	0.2763	0.4843	0.7609	1.0132	1.0069	1.0604
16.7256	9.2974	0.4321	0.6746	1.4228	1.0895	1.0529	0.8712
4.0365	22.5839	0.0814	0.1555	0.1673	0.9818	1.0478	1.1368
8.2800	23.0360	0.1520	0.2705	0.3109	1.0055	1.0720	1.1998
15.9804	21.4290	0.2527	0.4383	0.6086	1.0120	0.9919	0.9977
3.9848	29.9892	0.0604	0.1161	0.1269	0.9713	1.0277	0.9755
7.8453	30.8065	0.1128	0.2147	0.2379	0.9781	0.9923	1.0255
18.3816	36.1604	0.2114	0.3718	0.4010	1.0966	1.1179	2.9330
<i>Relative Anaplerosis: y = 0.1</i>							
4.1090	9.2983	0.1959	0.3369	0.3254	0.9401	1.0007	1.6351
8.1558	9.1968	0.3032	0.5172	0.7695	1.0332	0.9914	1.1044
16.6428	9.3471	0.4519	0.7055	1.4382	1.1014	1.0093	0.8641
4.1193	23.1058	0.0880	0.1671	0.1669	1.0147	0.9961	1.2171
7.7625	21.4432	0.1622	0.2884	0.2961	0.9555	0.9403	1.2170
15.6492	21.3362	0.2711	0.4429	0.5933	0.9958	0.9697	1.0037
4.1918	32.6719	0.0677	0.1248	0.1180	0.9986	1.0135	1.1196
8.5698	32.9991	0.1176	0.2139	0.2602	1.1086	1.0818	1.0470
16.8912	32.8816	0.2174	0.3657	0.4500	1.0563	1.0395	1.0554
<i>Relative Anaplerosis: y = 0.3</i>							
3.8709	8.2339	0.1996	0.3030	0.3944	0.9741	1.0224	1.2898
8.8389	9.5217	0.3478	0.4712	0.6996	1.0676	1.1493	1.3295
17.7606	10.1937	0.4874	0.6694	1.4377	1.1934	1.1535	0.9271
3.9641	21.4057	0.0958	0.1760	0.1812	1.0070	0.9016	1.2126
8.1765	22.4570	0.1761	0.2755	0.3337	1.0373	1.0347	1.2094
16.8084	22.8293	0.2975	0.4413	0.6441	1.0735	1.0431	0.9866
3.9020	30.2422	0.0819	0.1292	0.1134	0.8673	0.9101	1.1486
7.8660	30.5001	0.1367	0.2213	0.2354	0.9825	0.9618	1.0929
16.3116	32.1821	0.2282	0.3777	0.4828	1.0805	0.9777	0.9506

Actual values of v_{CAC} and v_{TA} were obtained from examining the input to the simulation runs; actual values of L_{C-2} , L_{C-4} and L_{C-2B} were obtained from curve fitting by MLAB; v_{CAC} and v_{TA} are in units of $\mu\text{mol/g dry wt}\cdot\text{min}$. L_{C-2} , L_{C-4} , and L_{C-2B} are in units of min^{-1} . Predicted values of L_{C-2B} were obtained by use of Eq. (23), as described in the text. Included are estimates of L_{C-2} and L_{C-4} , for comparison (these were previously reported in Table 3 of Cohen and Bergman, 1995).

3.3.2. The magnitude of the dominant rate constant for a pool in a metabolic cycle differs substantially from the fractional turnover rate of any given pool in the cycle

Indeed, it can be shown that the complete set of rate constants in an acyclic metabolic pathway is changed if the pathway is made into a cyclic pathway. We illustrate this for three pools having masses M_1 , M_2 and M_3 , and fractional enrichments x_1 , x_2 , and x_3 , respectively (Fig. 8). For illustration, we assume that the set of rate constants of $p(t)$ does not contain the dominant rate constant.¹⁴ The result of this analysis (see derivation of Eq. (A.39) in the Appendix) is that

$$\lambda_{\text{dom}} \approx \lambda_{\text{max}} w_0 / (w_0 + w_3),$$

¹⁴Mathematically, we assume that the magnitude of the dominant rate constant of $p(t)$ is greater than the magnitude of the dominant rate constant of $x_i(t)$, for $i = 1, 2$, and 3 (i.e. $p(t)$ changes faster than any of the pools in Fig. 8).

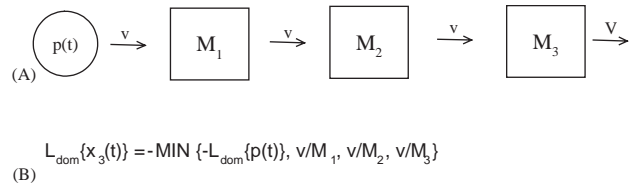


Fig. 7. Relation of dominant rate constant of a catenary system of metabolic pools to the fractional turnover rate of each pool. (A) Diagram of metabolic pathway. (B) Mathematical formula for the dominant rate constant of the fractional enrichment $x_3(t)$ of pool 3, where $L_{\text{dom}}\{\cdot\}$ denotes the operation of finding the dominant rate constant of a time-varying function; $\text{MIN}\{\cdot\}$ denotes the operation of finding the minimum value of a set of values; M_i equals the moles of molecules in pool i , $i = 1, 2, 3$; v equals the rate of conversion from each pool to its immediate successor (mol/min); $x_i(t)$ denotes the fractional enrichment of pool i at time t ($i = 1, 2, 3$); and $p(t)$ equals the fractional enrichment of immediate precursor pool of pool 1, at time t .

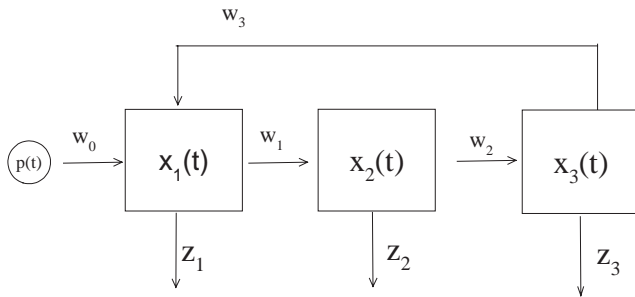


Fig. 8. Model of metabolic pathway with three pools. Pool i has mass M_i and fractional enrichment $x_i(t)$. The sole external source of ^{13}C is via the flux w_0 , having fractional enrichment $p(t)$ at time t . The fluxes w_1 , w_2 and w_3 equal the rate of conversion of M_1 to M_2 , M_2 to M_3 and M_3 to M_1 , respectively. We assume that each pool has a flux z_i directed towards metabolic pools other than those in this cycle and that each of the rates of flux (w_i , $i = 0, 1, 2, 3$ and z_i , $i = 1, 2, 3$) is constant over time.

where λ_{dom} is the dominant rate constant, λ_{max} is the maximum of λ_1 , λ_2 and λ_3 , and λ_i equals the negative of the fractional turnover rate of the pool having mass M_i , for $i = 1, 2$, and 3 .

3.3.3. Rate constants can be transmitted through a metabolic pathway

Examination of the equations for the dominant rate constant (Figs. 7 and 8) reveals the dependence on L_{DOM} on the fractional turnover rates of preceding pools. In this manner, slower rate constants affect the dynamics of succeeding pools.

3.3.4. Fractional turnover rates, coefficients of exponential terms, and rate constants of adjoining metabolic pools are related by simple mathematical formulas

Suppose pool 1 is the unique precursor of pool 2 that contains any isotopic label and let $x_1(t)$ and $x_2(t)$ denote the fractional enrichments of pool 1 and pool 2, respectively (Fig. 1). The rate constant λ_j (common to both pools) and the fractional turnover rate (v_2/M_2) of pool 2 are related to a_j and b_j , the coefficients of $e^{\lambda_j t}$ in $x_1(t)$ and $x_2(t)$, respectively, by the following formula (see appendix, Eq. (A.53)):

$$\lambda_j = (v_2/M_2)\{(a_j/b_j)(v_1/v_2) - 1\}$$

assuming that $\lambda_j \neq -v_2/M_2$.¹⁵

3.3.5. Coefficients of exponential terms are flux-weighted averages of enrichments of precursor pools

We illustrate this concept in the case of a single pool, in Fig. 1. From Eq. (11), it can be seen that at each time

¹⁵We have shown (see appendix) that the magnitude of the dominant rate constant for a metabolic cycle differs from the fractional turnover rate of any pool in the cycle. Hence, if λ_j is the dominant rate constant for a cyclic pathway, then $\lambda_j \neq -v_2/M_2$ and Eq. (A.53) applies.

point (assuming metabolic steady state holds), the magnitude of the enrichment of pool 2 is proportional to the fraction of influx into pool 2 that comes from pool 1 (i.e., v_1/v_2) and to the enrichment (p) of pool 1. This is extended to multiply labeled precursor pools in the appendix.

3.3.6. Obtaining a formula for the dominant rate constant is easiest when one metabolic pool is much larger than the others

In the appendix, we demonstrate mathematically that when the rate constants have very different magnitudes, the formulas for dominant rate constant have a particularly simple form (Eq. (A.78)).

4. Discussion

We propose the dominant rate constant as a concept with which to describe, analyze and reason about the relationship of the dynamics of isotopic enrichment atoms within metabolic intermediates to metabolic flux rates and pool sizes. Measurement of dominant rate constants can be performed by fitting the graph of isotopic enrichment versus time to a sum of exponential terms (Eq. (12)), yielding a quantitative indicator of the most slowly changing component of the isotopic enrichment.¹⁶ This simple procedure obtains regardless of the number of differential equations used to completely describe the system and regardless of the complexity of the diagram describing the connectivity among metabolic pools.¹⁷ Mathematical analysis of metabolism using the dominant rate constant can be used to obtain an algebraic formula for the flux through a metabolic pathway, as we have illustrated for the citric acid cycle in heart (Eq. (19)), for the glycolytic pathway in brain (Eq. (21)), and for glutamine synthesis in brain (Eq. (22)). These formulas do not depend upon the steady state enrichment or even upon the attainment of isotopic steady state of the precursor pools, provided that the dominant rate constant can be estimated.

4.1. Analysis of models of cardiac metabolism

Examination of formulas used to estimate relative anaplerosis in heart at isotopic steady state led us to

¹⁶Even though the system of ordinary differential equations describing the time course of enrichment of metabolites in a given metabolic pathway may in theory yield a solution consisting of a sum of 6 or more exponential terms, in practice at most 2 terms (often only 1 term) can be resolved from the data. Our theory relates the exponent that can be most readily obtained from the data to the rate of metabolic flux.

¹⁷In these analyses, we assume that metabolic steady state holds, so that pool sizes and flux rates are not changing (whereas atomic enrichments may be changing).

point out the importance of the isotopic enrichment of pyruvate that is substrate for anaplerotic reactions (Cohen and Bergman, 1997). In the current investigation, we examined the use of dominant rate constants for estimation of the rate v_{CAC} of the citric acid cycle and the rate v_{TA} of the transaminase reactions during conditions in which the pyruvate becomes labeled (e.g., from metabolism of $[1-^{13}\text{C}]\text{glucose}$ to $[3-^{13}\text{C}]\text{pyruvate}$). By mathematical analysis and computer simulation, we derived and tested formulas for v_{CAC} and v_{TA} that hold even during isotopic labeling of anaplerotic pyruvate (see Eqs. (19) and (20)). As predicted from our analysis of dominant rate constants in general, the dominant rate constant ($L_{\text{C-4}}$) of C-4 of glutamate decreases with the fractional turnover rate ($\text{FTR}_{\text{pyruvate}}$) of pyruvate that is substrate for anaplerosis (Fig. 4). Surprisingly, for values of $\text{FTR}_{\text{pyruvate}}$ below a certain value, the relationship of $\ln(\text{FTR}_{\text{pyruvate}})$ and $L_{\text{C-4}}$ of glutamate is approximately linear.

4.2. Analysis of models of cerebral glucose metabolism

In the estimation of cerebral glycolysis and citric acid cycle flux in laboratory animals (Rothman et al., 1985; Fitzpatrick et al., 1990) and in humans (Petroff et al., 1992; Rothman et al., 1992) using *in vivo* using NMR spectroscopy, $[1-^{13}\text{C}]\text{glucose}$ has been administered intravenously and the time course of enrichment of C-3 lactate and C-4 glutamate used to estimate glycolytic and citric acid cycle fluxes, respectively (Fig. 5).¹⁸ Isotopic enrichment of carbon C-3 of lactate (or C-4 of glutamate) as a function of time can be fitted with a function having a single exponential term:

$$y(t) = a_0(1 - e^{-\lambda t}). \quad (25)$$

To the best of our knowledge, no one has pointed out that if the lactate concentration exceeds the concentration of glutamate, then the dominant rate constants for C-2 and C-4 of glutamate will not reflect the turnover of label in glutamate but rather the fractional turnover rate of lactate.¹⁹ This reflects the properties of the dominant rate constant, because a similar problem will occur if the concentration of citrate (or of any other intermediate of the citric acid cycle) exceeds the concentration of glutamate.²⁰ A similar analysis of the dominant rate constant of the fractional enrichment of carbon C-3 of lactate (Fig. 5) shows that the fractional turnover rate

$\text{FTR}_{\text{lactate}}$ of lactate can be used to estimate the rate of conversion of glucose to lactate only if the fractional turnover rate $\text{FTR}_{\text{glucose}}$ of glucose exceeds the fractional turnover rate of lactate. In short, one needs $\text{FTR}_{\text{glucose}} > \text{FTR}_{\text{lactate}}$ in order for the fractional turnover rate of lactate to yield $v_{\text{glycolysis}}$; in addition, one needs $\text{FTR}_{\text{lactate}} > \text{FTR}_{\text{glutamate}}$ in order for the fractional turnover rate $\text{FTR}_{\text{glutamate}}$ of glutamate to yield v_{CAC} .

5. Conclusion

The dominant rate constant is a concept that enables one to analyze, reason about and discuss the dynamics of isotopic enrichment, especially with regard to the mathematical relationship to values of flux rates and pool sizes. In practice, applications of the dominant rate constant rely on the existence of one or more pools that are much larger than the remaining pools—exactly the situation *in vivo*, in which many pools are too small to be measured. Fluctuations in enrichments of precursor pools do not affect measurements of dominant rate constants (provided the fluctuations are much faster than the rate constant to be measured). We have derived specific algebraic formulas relating rates of flux in the citric acid cycle, glycolytic pathway, and glutamine synthetic pathway to the dominant rate constants of isotopic enrichment of individual carbons.

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Appendix A. Mathematical derivations

A.1. Laplace Transform

For completeness, we review the Laplace transform (Arfken and Weber, 1995; Hildebrand, 1962; Oppenheim and Willsky, 1983), which is useful for rewriting

¹⁸The C-3 of pyruvate is transferred to C-2 of acetyl CoA (the methyl carbon), and from there to C-4 of glutamate (Cohen and Bergman, 1995).

¹⁹Lactate concentration exceeding glutamate concentration has been reported in some brain cell preparations, e.g., neuronal soma enriched (Petroff et al., 1993) or synaptically enriched (Petroff et al., 1991) fractions of rat cerebrum.

²⁰This dependence on pool sizes was made explicit in our derivation of Eqs. (16)–(18).

differential equations as algebraic equations, from which the rate constants can be derived. The Laplace transform of a function $f(t)$ is a function $\tilde{f}(s)$ of the Laplace variable (s) and is defined as follows:

$$\tilde{f}(s) = \int_0^{\infty} f(t)e^{-st} dt \quad (\text{A.1})$$

For any function $f(t)$, we let $\mathbb{L}\{f\}$ denote the Laplace transform $\tilde{f}(s)$, and at other times, we may write \tilde{f} instead of $\tilde{f}(s)$. Transforms of some common functions are the following:

$$\mathbb{L}\{1\} = 1/s \text{ and } \mathbb{L}\{e^{kt}\} = 1/(s - k) \quad (\text{A.2, A.3})$$

The Laplace transform is a linear operator, i.e.,

$$\mathbb{L}\{f + g\} = \mathbb{L}\{f\} + \mathbb{L}\{g\} \text{ and } \mathbb{L}\{af\} = a\mathbb{L}\{f\} \quad (\text{A.4, A.5})$$

for any number a and any functions $f(t)$ and $g(t)$. An important transform for our purposes is that of the derivative of a function:

$$\mathbb{L}\{df/dt\} = s\tilde{f} - f(0) \quad (\text{A.6})$$

where $f(0)$ is the value of $f(t)$ at $t = 0$. In addition, the limiting value of $f(t)$ for large values of t (the steady state value), if it exists, can be obtained from its Laplace transform \tilde{f} :

$$\lim_{t \rightarrow \infty} \{f(t)\} = \lim_{s \rightarrow 0} \{s\tilde{f}(s)\}. \quad (\text{A.7})$$

Tables of functions and their Laplace transforms are common in physics and engineering texts (Arfken and Weber, 1995; Hildebrand, 1962; Oppenheim and Will-sky, 1983).

A.2. Properties of Dominant Rate Constants

A.2.1. The magnitude of the dominant rate constant for a pool in a metabolic cycle differs substantially from the fractional turnover rate of any given pool in the cycle

Suppose there are three pools having masses M_1 , M_2 and M_3 , and fractional enrichments x_1 , x_2 , and x_3 , respectively (Fig. 8). The fluxes w_1 , w_2 and w_3 equal the rate of conversion of M_1 to M_2 , M_2 to M_3 and M_3 to M_1 , respectively.²¹ We assume that each pool has a flux z_i directed towards metabolic pools other than the others in this cycle and that each of the rates of flux ($w_i, i = 0, 1, 2, 3$ and $z_i, i = 1, 2, 3$) are constant over time. For illustration, we assume that the sole source of ¹³C is via the flux w_0 , having fractional enrichment $p(t)$ at time t . The differential equations for the fractional enrichment $x_i(t)$ of the i th pool at time t are given below:

$$M_1 dx_1/dt = p(t)w_0 + x_3w_3 - x_1(w_1 + z_1), \quad (\text{A.8})$$

$$M_2 dx_2/dt = x_1w_1 - x_2(w_2 + z_2), \quad (\text{A.9})$$

$$M_3 dx_3/dt = x_2w_2 - x_3(w_3 + z_3). \quad (\text{A.10})$$

Taking the Laplace transform of these equations, and assuming

$$x_1(0) = x_2(0) = x_3(0) = 0$$

we obtain

$$sM_1\bar{x}_1 = \bar{p}w_0 + \bar{x}_3w_3 - \bar{x}_1(w_1 + z_1), \quad (\text{A.11})$$

$$sM_2\bar{x}_2 = \bar{x}_1w_1 - \bar{x}_2(w_2 + z_2), \quad (\text{A.12})$$

$$sM_3\bar{x}_3 = \bar{x}_2w_2 - \bar{x}_3(w_3 + z_3), \quad (\text{A.13})$$

where the Laplace transform of a variable is denoted by overhead bar (thus \bar{x}_1 is a function of the Laplace variable s and is the Laplace transform of $x_1(t)$). Solving these equations simultaneously for \bar{x}_3 , we obtain

$$\bar{x}_3(s) = \frac{w_0w_1w_2\bar{p}}{(s - \lambda_1)(s - \lambda_2)(s - \lambda_3)M_1M_2M_3 - w_1w_2w_3} \quad (\text{A.14})$$

where $\lambda_1(\lambda_2, \lambda_3)$ is the negative of the fractional turnover rate of M_1 (M_2, M_3 , respectively), assuming metabolic steady state, i.e., (Fig. 8):

$$\lambda_1 = -(w_1 + z_1)/M_1 = -(w_0 + w_3)/M_1, \quad (\text{A.15})$$

$$\lambda_2 = -(w_2 + z_2)/M_2 = -w_1/M_2, \quad (\text{A.16})$$

$$\lambda_3 = -(w_3 + z_3)/M_3 = -w_2/M_3. \quad (\text{A.17})$$

To obtain the rate constants $x_i(t)$ in Eqs. (A.8)–(A.10), we find the “poles” of $\bar{x}_i(s)$ in Eqs. (A.11)–(A.13), i.e., those values of the Laplace variable s that cause the $\bar{x}_i(s)$ to become infinite (Arfken and Weber, 1995). Setting the denominator in the expression for $\bar{x}_3(s)$ to zero, we obtain the characteristic equation for $x_3(t)$:

$$g(s) = (s - \lambda_1)(s - \lambda_2)(s - \lambda_3) - w_1w_2w_3/M_1M_2M_3 = 0 \quad (\text{A.18})$$

whose solution yields some of the rate constants for $x_3(t)$. The remainder of the rate constants are the poles of $\bar{p}(s)$, which (from Eq. (A.14)) are also poles of \bar{x}_3 . Thus the fractional enrichment of the precursor pool contributes to the set of rate constants for fractional enrichments of pools within a metabolic pathway. It is easily shown that $x_1(t)$ and $x_2(t)$ have the same characteristic equation as $x_3(t)$.

To show that the dominant rate constant of the cyclic pathway is different from that of the acyclic pathway (i.e., when $w_3 = 0$), we simply examine the solutions to Eq. (A.18), assuming that the precursor’s fractional enrichment changes are more rapid than those induced by the metabolic network in Fig. 8 (See footnote 14). We shall view Eq. (A.18) as a perturbation of Eq. (A.19):

$$f(s, \{\lambda_i\}) = 0, \quad (\text{A.19})$$

²¹We assume that w_i and M_i are in compatible units, i.e., kg/min or mol/min (for w_i) and kg or mol, respectively, for M_i .

where

$$f(s, \{\lambda_i\}) = (s - \lambda_1)(s - \lambda_2)(s - \lambda_3), \quad (\text{A.20})$$

$$f(s, \{\lambda_i\}) = s^3 - C_1 s^2 + C_2 s - C_3, \quad (\text{A.21})$$

whose solutions are λ_1 , λ_2 and λ_3 (the negative values of the turnover rates of pools 1, 2 and 3, resp.), and where

$$C_1 = \lambda_1 + \lambda_2 + \lambda_3, \quad (\text{A.22})$$

$$C_2 = \lambda_1 \lambda_2 + \lambda_2 \lambda_3 + \lambda_3 \lambda_1, \quad (\text{A.23})$$

$$C_3 = \lambda_1 \lambda_2 \lambda_3. \quad (\text{A.24})$$

Let us regard the solutions to Eq. (A.18) as

$$\lambda_1 + \Delta\lambda_1, \quad \lambda_2 + \Delta\lambda_2, \quad \text{and} \quad \lambda_3 + \Delta\lambda_3$$

which result in the following perturbations of C_1 , C_2 , and C_3 :

$$\Delta C_1 = (\lambda_1 + \Delta\lambda_1) + (\lambda_2 + \Delta\lambda_2) + (\lambda_3 + \Delta\lambda_3) - C_1, \quad (\text{A.25})$$

$$\Delta C_2 = (\lambda_1 + \Delta\lambda_1)(\lambda_2 + \Delta\lambda_2) + (\lambda_2 + \Delta\lambda_2)(\lambda_3 + \Delta\lambda_3) + (\lambda_3 + \Delta\lambda_3)(\lambda_1 + \Delta\lambda_1) - C_2, \quad (\text{A.26})$$

$$\Delta C_3 = (\lambda_1 + \Delta\lambda_1)(\lambda_2 + \Delta\lambda_2)(\lambda_3 + \Delta\lambda_3) - C_3. \quad (\text{A.27})$$

Comparison of Eqs. (A.25)–(A.27) with Eq. (A.18) shows that

$$\Delta C_1 = 0, \quad (\text{A.28})$$

$$\Delta C_2 = 0, \quad (\text{A.29})$$

$$\Delta C_3 = w_1 w_2 w_3 / (M_1 M_2 M_3). \quad (\text{A.30})$$

It can be shown²² that

$$dC_3/d\lambda_1 = (\lambda_2 - \lambda_1)(\lambda_3 - \lambda_1). \quad (\text{A.31})$$

with similar equations holding for $dC_3/d\lambda_2$ and $dC_3/d\lambda_3$. Next we assume that pool 1 has the smallest fractional turnover rate ($-\lambda_1$) among all of the pools (analogous proofs hold for pools 2 and 3). To finish the analysis, we use a first order Taylor series approximation of the change in C_3 induced by perturbation of λ_1 :

$$\Delta C_3 \approx (dC_3/d\lambda_1)\Delta\lambda_1. \quad (\text{A.32})$$

In order to derive a conveniently simple algebraic expression, we assume that the two smallest fractional turnover rates of the metabolic pools are widely separated (i.e., $0 > \lambda_1 \gg \lambda_2$ and $\lambda_1 \gg \lambda_3$); then from

²²Take the derivative of C_3 with respect to λ_1 , assuming that C_1 and C_2 are constant and that C_3 (as well as λ_2 and λ_3) is an implicit function of λ_1 . Eq. (A.31) can be generalized to an arbitrary number of pools, provided that Eq. (A.18) (suitably generalized) is the characteristic equation. Proof is by induction on the number of pools. Alternatively, for arbitrary n , write $\partial f_n(s, \{\lambda_i\})/\partial \lambda_1 = -f_n(s, \{\lambda_i\})/(s - \lambda_1) = -\prod_{j=2}^n (s - \lambda_j)$, which follows from Eq. (A.20), and $f_n(s, \{\lambda_i\})/\partial \lambda_1 = (-1)^n \partial C_n(\{\lambda_i\})/\partial \lambda_1$, which follows from Eq. (A.21), and then choose $s = \lambda_1$.

Eqs. (A.31) and (A.24) it follows that

$$dC_3/d\lambda_1 \approx \lambda_2 \lambda_3, \quad (\text{A.33})$$

$$dC_3/d\lambda_1 \approx C_3/\lambda_1. \quad (\text{A.34})$$

Eqs. (A.32) and (A.34) imply that

$$\Delta\lambda_1 \approx \Delta C_3 \lambda_1 / C_3. \quad (\text{A.35})$$

Substituting Eqs. (A.16), (A.17), (A.24) and (A.30) into Eq. (A.35) yields

$$\Delta\lambda_1 \approx w_3 / M_1. \quad (\text{A.36})$$

From Eqs. (A.15) and (A.36), the formula for the approximation of the dominant rate constant λ_{dom} for Eq. (A.18) equals

$$\lambda_{\text{dom}} \approx \lambda_1 + \Delta\lambda_1, \quad (\text{A.37})$$

$$\lambda_{\text{dom}} \approx -w_0 / M_1, \quad (\text{A.38})$$

$$\lambda_{\text{dom}} \approx \lambda_{\text{max}} w_0 / (w_0 + w_3), \quad (\text{A.39})$$

where λ_{max} is the maximum of λ_1 , λ_2 and λ_3 ($\lambda_{\text{max}} < 0$). Note that $w_3 \neq 0$, since we assume a metabolic cycle exists in Fig. 8.²³ Thus we have derived an approximate formula for the dominant rate constant and illustrated that the dominant rate constant for a metabolic cycle differs substantially from the fractional turnover rate of each pool of the cycle.²⁴

A.2.2. Fractional turnover rates, coefficients of exponential terms, and rate constants of adjoining metabolic pools are related by simple mathematical formulas

We now derive mathematical formulas relating the coefficients of exponential terms in $x_i(t)$, rate constants and fractional turnover rates of adjacent metabolic pools (Fig. 1).²⁵ Writing the equation for the fractional enrichments $x_1(t)$ and $x_2(t)$ of pools 1 and 2 (assuming metabolic steady state):

$$M_1 dx_1/dt = p w_1 - x_1 v_1, \quad (\text{A.40})$$

$$M_2 dx_2/dt = x_1 v_1 - x_2 v_2 \quad (\text{A.41})$$

and taking Laplace transforms:

$$M_1 (s \bar{x}_1 - x_1(0)) = \bar{p} w_1 - \bar{x}_1 v_1 \quad (\text{A.42})$$

²³Note that the formula for λ_{dom} remains correct for the open-loop case, i.e., when $w_3 = 0$.

²⁴More generally, the dominant rate constant can be estimated directly from the characteristic polynomial $f(x) = 0$ (of degree n) by observing that $f'(0)/f(0) = -\sum_{i=1}^n 1/\lambda_i$, where $\{\lambda_i, 1 \leq i \leq n\}$ is the set of roots of $f(x)$; cf Eqs. (A.21), (A.23) and (A.24). This formula can be used to derive Eq. (A.76) from Eq. (A.75) (below). In general, the method of moments (Lanczos, 1988) can be used to derive the following, for all $m > 1$: $d^{(m-1)}/dx^{(m-1)}\{f'(x)/f(x)\}_{x=0} = -(m-1)! \sum_{i=1}^n 1/(\lambda_i)^m$ from which $(1/\lambda_1)^m$ can be estimated.

²⁵Pool 1 is the unique isotopically enriched predecessor of pool 2 in this example.

$$M_2(s\bar{x}_2 - x_2(0)) = \bar{x}_1 v_1 - \bar{x}_2 v_2 \quad (\text{A.43})$$

yields the following:

$$\bar{x}_1 = (\bar{p}_1 w_1 + M_1 x_1(0)) / (sM_1 + v_1), \quad (\text{A.44})$$

$$\bar{x}_2 = (\bar{x}_1 v_1 + M_2 x_2(0)) / (sM_2 + v_2). \quad (\text{A.45})$$

Assume that the rate constants of $p(t)$ are much faster than the fractional turnover rates of pools 1 and 2. From Eqs. (12) and (13) (Section 2), we know the form of $x_1(t)$, $x_2(t)$, $\bar{x}_1(s)$ and $\bar{x}_2(s)$:

$$x_1(t) = a_0 + \sum_i a_i e^{\lambda_i t}, \quad (\text{A.46})$$

$$x_2(t) = b_0 + \sum_i b_i e^{\lambda_i t}, \quad (\text{A.47})$$

$$\bar{x}_1(s) = \frac{a_0}{s} + \sum_i \frac{a_i}{s - \lambda_i}, \quad (\text{A.48})$$

$$\bar{x}_2(s) = \frac{b_0}{s} + \sum_i \frac{b_i}{s - \lambda_i}, \quad (\text{A.49})$$

where $\lambda_i < 0$ and $\lambda_i \neq \lambda_j$ for $i \neq j$. Next we investigate the relationship between a_j and b_j , the coefficients of the same exponential term $e^{\lambda_j t}$ in $x_1(t)$ and $x_2(t)$, respectively. From Eqs. (14), (15), (A.48) and (A.49), we infer the following:

$$a_j = \lim_{s \rightarrow \lambda_j} \{(s - \lambda_j) \bar{x}_1(s)\} \quad (\text{A.50})$$

$$b_j = \lim_{s \rightarrow \lambda_j} \{(s - \lambda_j) \bar{x}_2(s)\} \quad (\text{A.51})$$

Using Eqs. (A.45), (A.50) and (A.51), we can relate the rate constant λ_j and the fractional turnover rate of pool 2 to the coefficient of $e^{\lambda_j t}$ in $x_1(t)$ and $x_2(t)$:

$$b_j = a_j v_1 / (\lambda_j M_2 + v_2) \quad (\text{A.52})$$

or equivalently,

$$\lambda_j = (v_2 / M_2) \{(a_j / b_j)(v_1 / v_2) - 1\} \quad (\text{A.53})$$

assuming that the denominator in Eq. (A.52) is nonzero, i.e., $\lambda_j \neq -v_2 / M_2$.

A.2.3. Coefficients of exponential terms are flux-weighted averages of enrichments of precursor pools

Consider Fig. 9, in which three pools of masses M_1 , M_2 , and M_3 are the immediate metabolic precursors of a pool of mass M_0 , whose fractional enrichment is measured as a function of time. For simplicity, we will assume that there is a constant enrichment p_i feeding into the pool of mass M_i , $i = 1, 2, 3$ and that the system is at metabolic steady state (the pool sizes and flux rates unchanging over time). The differential equations for fractional enrichments $x_i(t)$ are the following:

$$M_i dx_i/dt = p_i v_i - x_i v_i \text{ for } i = 1, 2, 3 \quad (\text{A.54, A.55, A.56})$$

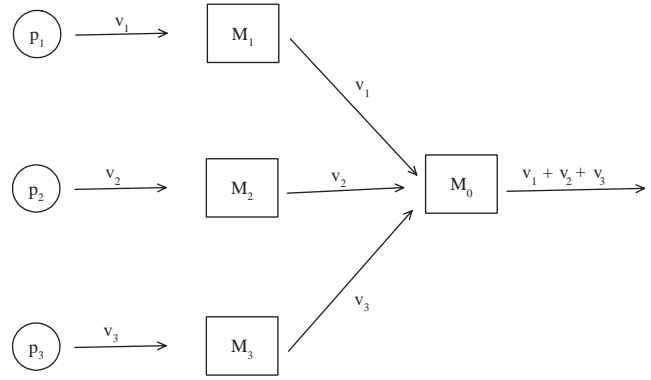


Fig. 9. Model of three metabolic pools (having masses M_1 , M_2 and M_3) which are immediate precursors of a fourth pool (having mass M_0). The fractional enrichment of the immediate precursor to the pool of mass M_i is p_i , for $i = 1, 2$, and 3.

$$M_0 dx_0/dt = x_1 v_1 + x_2 v_2 + x_3 v_3 - x_0(v_1 + v_2 + v_3) \quad (\text{A.57})$$

(cf Eqs. (5) and (11)). Assuming $x_i(0) = 0$, for $i = 0, 1, 2, 3$, we obtain:

$$\bar{x}_i(s) = p_i/s - p_i/(s + v_i/M_i) \text{ for } i = 1, 2, 3 \quad (\text{A.58, A.59, A.60})$$

$$\bar{x}_0(s) = (v_1 \bar{x}_1 + v_2 \bar{x}_2 + v_3 \bar{x}_3) / (sM_0 + v_1 + v_2 + v_3), \quad (\text{A.61})$$

where, for clarity, the dependence of \bar{x}_i ($i = 1, 2, 3$) in Eq. (A.61) on s is suppressed. Substitution of Eqs. (A.58)–(A.60) into Eq. (A.61) and expanding in partial fractions (Hildebrand, 1962; Oppenheim and Willsky, 1983), one can discern two cases that exhibit a satisfying result:

A.2.3.1. Case 1. If the fractional turnover rate of the pool of mass M_0 is much *greater* than that of each of the pools of mass M_1 , M_2 , and M_3 (which implies that M_0 is much smaller than M_1 , M_2 or M_3), then

$$x_0(t) \approx a_0 + a_1 e^{\lambda_1 t} + a_2 e^{\lambda_2 t} + a_3 e^{\lambda_3 t}, \quad (\text{A.62})$$

where

$$a_0 = (v_1 p_1 + v_2 p_2 + v_3 p_3) / (v_1 + v_2 + v_3), \quad (\text{A.63})$$

$$a_i = -v_i p_i / (v_1 + v_2 + v_3), \quad (\text{A.64, A.65, A.66})$$

and $\lambda_i = -v/M_i$ for $i = 1, 2, 3$.

A.2.3.2. Case 2. If the fractional turnover rate of the pool of mass M_0 is much *less* than that of each of the pools of mass M_1 , M_2 , and M_3 (which implies that mass M_0 is much greater than any one of M_1 , M_2 or M_3), then

$$x_0(t) \approx a_0(1 - e^{\lambda_1 t}) \quad (\text{A.67})$$

where

$$a_0 = (v_1 p_1 + v_2 p_2 + v_3 p_3) / (v_1 + v_2 + v_3), \quad (\text{A.68})$$

$$\lambda_1 = -(v_1 + v_2 + v_3)/M_0. \quad (\text{A.69})$$

This example illustrates that large disparities in the fractional turnover rates of metabolic pools have strong and specific effects on the dynamics of enrichment of the pools (both with respect to rate constants as well as coefficients).

A.2.4. Obtaining a formula for the dominant rate constant is easiest when one metabolic pool is much larger than the others

We illustrate how the mathematics favors widely separated pool sizes, by considering two exchanging pools (Fig. 6). We write the differential equations (suppressing the dependence on time t of $p_1, p_2, x_1, x_2, dx_1/dt$ and dx_2/dt):

$$M_1 dx_1/dt = v_0 p_1 + (v_1 - v_2 + v_3)x_2 - (v_0 + v_1 - v_2 + v_3)x_1, \quad (\text{A.70})$$

$$M_2 dx_2/dt = v_3 p_2 + v_1 x_1 - (v_1 + v_3)x_2. \quad (\text{A.71})$$

Taking Laplace transforms and solving (cf Eqs. (A.8)–(A.14)), we obtain

$$\bar{x}_1 = (v_0 \bar{p}_1 + (v_1 - v_2 + v_3) \bar{x}_2) / (sM_1 + v_0 + v_1 - v_2 + v_3), \quad (\text{A.72})$$

$$\bar{x}_2 = (v_3 \bar{p}_2 + v_1 \bar{x}_1) / (sM_2 + v_1 + v_3) \quad (\text{A.73})$$

and, finally,

$$\bar{x}_2 = (v_0 v_1 \bar{p}_1 + v_3 \bar{p}_2 (sM_1 + v_0 - v_2 + v_3)) / D(s), \quad (\text{A.74})$$

where

$$D(s) = (sM_2 + v_1 + v_3)(sM_1 + v_0 + v_1 - v_2 + v_3) - v_1(v_1 - v_2 + v_3) = s^2 M_1 M_2 + s\{(v_1 + v_3)(M_1 + M_2) + (v_0 - v_2)M_2\} + v_3(v_0 + v_1 - v_2 + v_3) + v_0 v_1 \quad (\text{A.75})$$

The dominant rate constant of $x_1(t)$ and $x_2(t)$ is the maximum of the dominant rate constants of $p_1(t), p_2(t)$, and of the roots of $D(s) = 0$. In the following analysis we assume that the dominant rate constant of $x_2(t)$ is equal to the larger of the two roots of $D(s)$. We have proven (Cohen and Bergman, 1995) that if $b^2 - 4ac \gg 0$, then the larger of the two roots of $D(s) = as^2 + bs + c = 0$ is approximately equal to $-c/b$. Using Eq. (A.75), the formula is

$$\lambda_{\text{dom}} \approx \frac{-\{v_3(v_0 + v_1 - v_2 + v_3) + v_0 v_1\}}{\{(v_1 + v_3)(M_1 + M_2) + (v_0 - v_2)M_2\}} \quad (\text{A.76})$$

provided that

$$\{(v_1 + v_3)(M_1 + M_2) + (v_0 - v_2)M_2\}^2 - 4M_1 M_2 \{v_3(v_0 + v_1 - v_2 + v_3) + v_0 v_1\} \gg 0. \quad (\text{A.77})$$

It is easily shown²⁶ that this inequality (Eq. (A.77)) is satisfied if $M_1 \ll M_2$ and either

(1) $v_3 \ll v_0$; or (2) $v_3 \ll v_1$; or (3) $v_3 = 0$. In any case, Eq. (A.76) yields

$$\lambda_{\text{dom}} \approx -v_3/M_2 - v_0 v_1 / (M_2(v_0 + v_1 - v_2 + v_3)) \quad (\text{A.78})$$

and we observe that $v_0 - v_2 + v_3 \geq 0$ and $v_1 - v_2 + v_3 \geq 0$ (see Fig. 6).

A.2.4.1. Applications of Eq. (A.78). If $v_3 = 0$, then having a large difference in M_1 and M_2 suffices to enable the use of Eq. (A.78). For the special case in which $v_0 = v_{\text{CAC}}$, $v_1 = v_{\text{TA}}$, $v_2 = 0$, $p(t)$ = fractional enrichment of C-2 of acetyl CoA, x_1 = fractional enrichment of C-4 of α -ketoglutarate, x_2 = fractional enrichment of C-4 of glutamate, and M_2 = mass of glutamate, Eq. (A.78) provides an estimate of the magnitude $L_{\text{C-4}}$ of the dominant rate constant for C-4 of glutamate in heart:

$$L_{\text{C-4}} \approx v_{\text{CAC}} v_{\text{TA}} / \{(v_{\text{CAC}} + v_{\text{TA}})[\text{glutamate}]\} \quad (\text{A.79})$$

in agreement with a previously derived formula (Eq. (6) in Cohen and Bergman, 1995), assuming $[\alpha\text{-ketoglutarate}] \ll [\text{glutamate}]$.

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²⁶It suffices to have $(v_0 + v_1 - v_2 + v_3)/(v_0 + v_3) \gg 4M_1/M_2$.

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