Evaluation of the pentose phosphate pathway from $^{14}\text{CO}_2$ data

Fallibility of a classic equation when applied to non-homogeneous tissues

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A classic equation that has frequently been used to estimate the fraction of glucose metabolized by the pentose phosphate pathway, using $^{14}\text{CO}_2$ data, is more simply re-derived with careful consideration of the assumptions involved and the conditions under which it is applicable. The equation is shown to be unreliable for non-homogeneous tissues, depending on the fraction of triose phosphate converted to CO_2 . The formula in question is as follows:

$$([1]\text{CO}_2/G - [6]\text{CO}_2/G) / (1 - [6]\text{CO}_2/G) = 3F_{\text{met.}} / (1 + 2F_{\text{met.}})$$

where $[1]\text{CO}_2$ and $[6]\text{CO}_2$ are output rates of carbons 1 and 6 of glucose respectively to CO_2 , G is the rate of glucose uptake and $F_{\text{met.}}$ is the fraction of the glucose that is metabolized to CO_2 and triose phosphate by the pentose phosphate pathway, allowing for recycling of an appropriate fraction of the fructose-6-phosphate produced by the pathway. This analysis illustrates the importance of suitably testing any equation that assumes homogeneity before application to non-homogeneous tissues.

INTRODUCTION

In 1963, Katz & Wood [1], and Wood *et al.* [2] presented the following equation for estimating the fraction of glucose metabolized by the pentose phosphate pathway:

$$\frac{[1]\text{CO}_2/G - [6]\text{CO}_2/G}{1 - [6]\text{CO}_2/G} = \frac{3F_{\text{met.}}}{1 + 2F_{\text{met.}}} \quad (1)$$

Here $[1]\text{CO}_2$ and $[6]\text{CO}_2$ are the rates of output to CO_2 of carbons 1 and 6 respectively of glucose, as measured using $[1-^{14}\text{C}]\text{glucose}$ and $[6-^{14}\text{C}]\text{glucose}$, G is the rate of glucose uptake, and $F_{\text{met.}}$ is the fraction of the glucose taken up by the tissue that is metabolized to CO_2 and triose phosphate (triose- P) by the pentose phosphate pathway. The equation is still being used (e.g. refs. [3–9]), generally with no statement of the assumptions implicit in its use, and was recently reaffirmed by one of the original authors, with no warning about its limited applicability [10].

In developing eqn. (1) it was implicitly assumed that the tissue is homogeneous, at least to the extent that all cells are identical with respect to the relevant aspects of glucose metabolism. This permitted single values to be used for several variables, including the specific activities and reaction rates of various intracellular intermediates. It is the major purpose of the present paper to show that the above equation can be seriously in error when applied to non-homogeneous tissues.

Eqn. (1) depends on many simplifying assumptions in addition to that of homogeneity, including those that the tissue is (a) in an isotopic steady state and (b) non-gluconeogenic; these assumptions have frequently been ignored by investigators who have employed the equation. Moreover, it has not been generally recognized that the isotopic steady state can sometimes take considerable time to achieve, as signified by $^{14}\text{CO}_2$ output, especially from $[6-^{14}\text{C}]\text{glucose}$ [11,12].

Because the major purpose of the present paper is to examine the applicability of eqn. (1) to non-homogeneous tissues, it will be assumed that metabolism through the pentose phosphate pathway conforms to the 'classical' pathway, which was also assumed in the original derivation of eqn. (1). Thus no consideration will be given to the 'L-type' pathway recently proposed for certain tissues by Williams and his colleagues [13–15], which at present seems to be controversial ([16], and references therein).

FORMULATION FOR HOMOGENEOUS TISSUES

Eqn. (1) is re-derived in the Appendix by a different and in some ways simpler approach than that given by the original authors. This approach yields a formula for the fraction of glucose entering the pentose phosphate pathway as well as the fraction metabolized to CO_2 and triose- P by the pathway, and uses a different treatment of the effects of recycling on the metabolized fraction. The reactions to be considered are shown in Fig. 1. The assumptions needed for the derivations are as follows.

1. The tissue is homogeneous

This is assumed in the sense that glucose metabolism is quantitatively identical in all cells. This permits assignment of single values to various intracellular variables, such as the specific activity of the hexose- P pool and the fractionation of metabolism of glucose-6- P (Glc-6- P) and triose- P between different pathways. This assumption was not stated specifically in the original derivation of eqn. (1), but was implicit in the assignment of single values to items of intermediary metabolism.

2. The tissue is in a metabolic and isotopic steady state

This means that the concentrations and specific activities of the intermediates of glucose metabolism and the flux rates between them are constant. The isotopic steady state would be achieved experimentally by keeping the specific activity of the glucose in the medium constant for a sufficient length of time before starting measurements. This is needed so that the measurements will determine the steady-state rates, on which the formulation is based.

3. Stoichiometric details of the pentose phosphate pathway

(a) It is assumed that the rates of synthesis of ribose and other possible products of the pathway are quantitatively negligible, except for CO_2 , triose- P and fructose 6-phosphate (Fru-6- P). (b) Each mol of Glc-6- P entering the pathway forms 1 mol of CO_2 containing only C-1 of Glc-6- P . (c) Each mol of Glc-6- P entering the pathway also forms $\frac{1}{2}$ mol of glyceraldehyde- P , containing only carbons 4, 5 and 6 of Glc-6- P , and $\frac{2}{3}$ mol of Fru-6- P , with the carbons of Glc-6- P rearranged in the orders 2-3-2-4-5-6- P and 2-3-3-4-5-6- P . Assumptions 3(b) and 3(c) are in accordance with the classical form of the pentose phosphate pathway (e.g. [2,17]) under conditions of assumption 3(a).

Fig. 1. Reactions considered in developing equations

Solid bars mark reactions assumed to be negligible in rate. Numbers in parentheses show distribution of carbon atoms of Glc-6-P among products of the pentose phosphate pathway. Numbers in square brackets show the distribution of the carbon atoms of Glc-6-P by the reactions of glycolysis and subsequent steps. Because of rearrangements in Fru-6-P produced by the pentose phosphate pathway, some C-2 of glucose appears in positions 1 and 3 of Glc-6-P and some C-3 in positions 1 and 2; however, important for this analysis, C-1 and C-6 of glucose do not appear in hexoses in other than their original positions. Abbreviations: DHAP, dihydroxyacetone-P; GAP, glyceraldehyde-P; G-6-P, glucose-6-P; F-6-P, fructose-6-P; ?, see assumption 5(a). For discussion of effects of possible reversibility of traffic from the pentose cycle to Fru-6-P, see assumption 5(b).

4. Disposition of glyceraldehyde-P and Fru-6-P produced by the pentose phosphate pathway: tissue not gluconeogenic

(a) Glyceraldehyde-P produced by the pentose phosphate pathway mixes freely with that formed in glycolysis and none is recycled into the pentose phosphate pathway. Non-recycling of this compound implies that the tissue is not gluconeogenic. (b) Fru-6-P produced by the pentose phosphate pathway mixes freely with that formed directly from Glc-6-P. (c) The hexose isomerase reaction is reversible and fast enough that Glc-6-P and Fru-6-P can be regarded as a single pool of hexose-P with uniform specific activity. Thus any difference between the specific activities of Glc-6-P and Fru-6-P, which tends to be caused by the recycling of Fru-6-P, is negligible.

5. Release of C-1 of Glc-6-P on entry into the pentose phosphate pathway is the only mechanism for altering the ratio of C-1 of glucose to C-6 of glucose in CO₂

(a) For example, glycerol-P formation might affect the C-1/C-6 ratio, because the fructoaldolase reaction delivers all of the C-1 and none of the C-6 of Glc-6-P to dihydroxyacetone-P, from which glycerol-P is formed. Therefore it is assumed either that glycerol-P formation is negligible in rate, or that the triose isomerase reaction is fast enough to make the C-1/C-6 ratio

essentially identical in dihydroxyacetone-P and glyceraldehyde-P, or that a combination of a relatively slow glycerol formation and a relatively fast isomerase reaction prevents the ratio in later 3-carbon products from being significantly affected by glycerol formation. (b) Another possibility is that there might be significant recurrent traffic through the transketolase and transaldolase reactions in the non-oxidative end of the pentose phosphate pathway from Fru-6-P, at least as far as xylulose-5-P and ribose-5-P and back to Fru-6-P. This would translocate some of the carbon atoms originally in the C-1 position of glucose and presumably alter the output of this carbon to CO₂. Wood *et al.* ([2], pp. 842–843) concluded that such recurrent traffic 'did not occur extensively' in their experiments, but it might occur significantly in some tissues under certain conditions. Indeed, Borowitz *et al.* [18] later found evidence for considerable recurrent traffic in *Tetrahymena*. However, this possibility was ignored by the original authors [1,2] in developing eqn. (1) and consequently will also be ignored in the present derivation.

6. Synthesis and degradation of glycogen are negligible

Results of assumptions

From the above assumptions it follows (see the Appendix) that the fraction of glucose that, after conversion to Glc-6-P, enters the pentose phosphate pathway in a homogeneous tissue is given by eqn. (A2b) in the Appendix:

$$F_{\text{ent.}} = \frac{[1]\text{CO}_2/\text{G} - [6]\text{CO}_2/\text{G}}{1 - [6]\text{CO}_2/\text{G}} \quad (2)$$

It thus turns out that the expression on the left of eqn. (1) represents the fraction entering the pentose phosphate pathway. It may also be noted that eqn. (2) for $F_{\text{ent.}}$ is independent of some assumptions that are listed above and are needed to estimate the fraction metabolized (considered below), such as certain details of the pentose phosphate pathway (assumption 3c) and the isotopic equilibration of Fru-6-P with Glc-6-P (assumptions 4b and 4c).

The fraction of glucose metabolized to CO₂ and triose-P in homogeneous tissues is given as follows by eqn. (A4a) in the Appendix:

$$F_{\text{met.}} = F_{\text{ent.}} / (3 - 2F_{\text{ent.}}) \quad (3a)$$

This can be solved for $F_{\text{ent.}}$ (eqn. A4b in the Appendix):

$$F_{\text{ent.}} = 3F_{\text{met.}} / (1 + 2F_{\text{met.}}) \quad (3b)$$

Using the last relation to eliminate $F_{\text{ent.}}$ from eqn. (2) yields eqn. (1) for the fraction metabolized, as given by the earlier authors [1,2].

Consequences and possible solutions when some of the assumptions are not strictly applicable are further considered in ref. [2].

FORMULATIONS FOR AN ILLUSTRATIVE NON-HOMOGENEOUS TISSUE

In order to determine whether the foregoing equations are applicable to a non-homogeneous tissue as well as to the homogeneous ones assumed in its derivation, a special case will be considered of a model tissue that contains two classes of cells, referred to as types a and b, occupying fractions W_a and W_b of the total weight of the tissue. Each class is assumed to meet all of the assumptions used in developing the relations for homogeneous tissues, but the classes differ from one another in rates of glucose uptake per unit weight (G_a and G_b respectively), in the fractions of glucose entering the pentose phosphate pathway ($F_{\text{ent.,a}}$ and $F_{\text{ent.,b}}$) and in the fraction of C-3 of glyceraldehyde-P that is

metabolized to CO_2 by the citric acid pathway (designated N_a and N_b). C-3 of triose- P is specified here because C-1 and C-6 of glucose reach only this position (next to the phosphate group) in triose- P .

True fraction entering the pentose phosphate pathway in the whole tissue

The true fraction of glucose entering the pentose phosphate pathway in the whole tissue is obviously the sum of the rates of entry into this pathway in the two cell types divided by the total rate of glucose uptake. Thus:

$$F_{\text{ent.,true}} = \frac{G_a W_a F_{\text{ent.,a}} + G_b W_b F_{\text{ent.,b}}}{G_a W_a + G_b W_b} \quad (4a)$$

Fraction entering the pentose phosphate pathway estimated by eqn. (2), neglecting non-homogeneity

From the values of G , W and $F_{\text{ent.}}$ assigned to the two cell types, the outputs of labelled CO_2 and the uptake of glucose can be determined for each cell type and summed to give the rates that would be observed experimentally on the whole tissue (see eqns. A5a, A5b and A5c in the Appendix). These observable rates can then be substituted into eqn. (2) to obtain the fraction that would be estimated from the experimental observations when ignoring the non-homogeneity of the tissue (eqn. A6 in the Appendix):

$$F_{\text{ent.,est.}} = \frac{G_a W_a F_{\text{ent.,a}}(1 - N_a) + G_b W_b F_{\text{ent.,b}}(1 - N_b)}{G_a W_a(1 - N_a) + G_b W_b(1 - N_b)} \quad (4b)$$

When $N_a = N_b$, eqns. (4a) and (4b) both reduce to the same value:

$$F_{\text{ent.,true}} = F_{\text{ent.,est.}} = \frac{G_a W_a F_{\text{ent.,a}} + G_b W_b F_{\text{ent.,b}}}{G_a W_a + G_b W_b}$$

When $F_{\text{ent.,a}} = F_{\text{ent.,b}}$, eqns. (4a) and (4b) both reduce to $F_{\text{ent.,a}}$:

$$F_{\text{ent.,true}} = F_{\text{ent.,est.}} = F_{\text{ent.,a}} = F_{\text{ent.,b}}$$

Thus the estimated value is correct under these two conditions, but is otherwise incorrect. Quantitative examples of the degree of error will be given below.

True fraction metabolized to CO_2 and triose- P by the pentose phosphate pathway in the whole tissue

The true fraction metabolized to CO_2 and triose- P by the whole tissue is obviously the sum of the rates by each class of cells divided by the total rate of glucose uptake:

$$F_{\text{met.,true}} = \frac{G_a W_a F_{\text{met.,a}} + G_b W_b F_{\text{met.,b}}}{G_a W_a + G_b W_b} \quad (5a)$$

Since each class of cells has been assumed to obey the equations for homogeneous tissues, their respective fractions metabolized to CO_2 and triose- P are given by eqn. (3a):

$$\begin{aligned} F_{\text{met.,a}} &= F_{\text{ent.,a}} / (3 - 2F_{\text{ent.,a}}) \\ F_{\text{met.,b}} &= F_{\text{ent.,b}} / (3 - 2F_{\text{ent.,b}}) \end{aligned}$$

Substituting these relations into eqn. (5a) yields an expression in terms of the defined properties of the model tissue:

$$F_{\text{met.,true}} = \frac{G_a W_a F_{\text{ent.,a}} / (3 - 2F_{\text{ent.,a}}) + G_b W_b F_{\text{ent.,b}} / (3 - 2F_{\text{ent.,b}})}{G_a W_a + G_b W_b} \quad (5b)$$

Fraction metabolized to CO_2 and triose- P estimated by eqn. (1), neglecting non-homogeneity

According to eqn. (3a), the value estimated by applying the equations for homogeneous tissues is:

$$F_{\text{met.,est.}} = F_{\text{ent.,est.}} / (3 - 2F_{\text{ent.,est.}})$$

This can be re-expressed in terms of the defined properties of the model tissue by using eqn. (4b) to eliminate $F_{\text{ent.,est.}}$, with the following result:

$$F_{\text{met.,est.}} = \frac{G_a W_a F_{\text{ent.,a}}(1 - N_a) + G_b W_b F_{\text{ent.,b}}(1 - N_b)}{G_a W_a(3 - 2F_{\text{ent.,a}})(1 - N_a) + G_b W_b(3 - 2F_{\text{ent.,b}})(1 - N_b)} \quad (5c)$$

When $F_{\text{ent.,a}} = F_{\text{ent.,b}}$, eqns. (5b) and (5c) both reduce to the same value:

$$F_{\text{met.,true}} = F_{\text{met.,est.}} = F_{\text{ent.,a}} / (3 - 2F_{\text{ent.,a}})$$

However, unlike the equations for entry of glucose into the pentose phosphate pathway, those for $F_{\text{met.,true}}$ and $F_{\text{met.,est.}}$ do not become equal for the special case $N_a = N_b$. Thus $F_{\text{met.,est.}}$ is incorrect under even more conditions than is $F_{\text{ent.,est.}}$. Quantitative examples will be given below.

QUANTITATIVE EXAMPLES OF ERRORS IN FRACTIONS CALCULATED FOR A NON-HOMOGENEOUS TISSUE WHEN USING EQUATIONS FOR HOMOGENEOUS TISSUES

In this section the true and estimated values for the model non-homogeneous tissue containing two types of cells, designated a and b, will be compared in a special case where the pentose phosphate pathway is inactive in the type b cells and 40% of the C-3 of glyceraldehyde- P is metabolized to CO_2 by these cells. Thus $F_{\text{ent.,b}} = 0$ and $N_b = 0.4$. For simplicity, it is further assumed that each class comprises half the weight of the tissue ($W_a = W_b = 0.5$) and that the rates of glucose uptake per unit weight are both 100 ($G_a = G_b = 100$).

In Fig. 2 the true and estimated values of the fraction entering the pentose phosphate pathway in the whole tissue are plotted against N_a , the fraction of C-3 of glyceraldehyde- P that is metabolized to CO_2 by the type a cells, using eqns. (4a) and (4b). This is done for several values of the fraction entering the pathway in these cells (indicated by ' $F_{\text{ent.,a}}$ ' at the top of the Figure). The same is done in Fig. 3 for the fraction metabolized to CO_2 and triose- P , using eqns. (5b) and (5c). It is apparent that the errors can be quite large, especially when the estimated fractions are low.

DISCUSSION

It has been shown that a classic equation (eqn. 1), which is frequently used to estimate the fraction of glucose metabolized by the pentose phosphate pathway, can be very inaccurate when applied to tissues that are not homogeneous with respect to intermediary metabolism of carbohydrate. This is true even when each of the constituent cell types obeys all of the assumptions used in deriving the equation. In fact, the equation is not infallible in indicating even the *direction* of change in activity of the pentose phosphate pathway between different states of the same non-homogeneous tissue. This is because apparent changes in activity of the pathway may instead be due to changes in the fraction of triose- P oxidized to CO_2 by the citric acid cycle (Figs. 2 and 3). Possibly the equation would be reliable for some purified cell preparations, although even there questions might arise about the quantitative equality of metabolism in histologically identical cells. Also, it must be remembered that errors other than those due to non-homogeneity will arise if the various assumptions on which the equations depend are not all fulfilled.

In the extreme case where all of C-3 in glyceraldehyde- P is converted to CO_2 in the cells with an active pentose phosphate pathway ($N_a = 1$ in Figs. 2 and 3), no evidence of the pathway is

Fig. 2. Comparison of the estimated fraction of glucose entering the pentose phosphate pathway (broken lines) with the true fraction (solid lines) in a model tissue

The tissue is assumed to contain two kinds of cells (types a and b), each constituting half the weight of the tissue and each consuming half the glucose taken up by the whole tissue. The type b cells have no pentose phosphate pathway activity and are assumed to convert 40% of C-3 of glyceraldehyde-P to CO_2 . The plot shows that the estimate for the whole tissue is highly dependent on the fraction of C-3 of glyceraldehyde-P converted to CO_2 in the type a cells (N_a). This relationship to N_a is shown for four different fractions of glucose entering the pentose phosphate pathway in these cells ($F_{\text{ent},a}$ values at the top of the Figure). The estimates are correct [estimated (Est.) = true] only in special cases where the fraction of glyceraldehyde-P converted to CO_2 is the same (40%) in the type a as in the type b cells. PC, pentose cycle (pentose phosphate pathway).

obtained from CO_2 measurements. This is because all of both C-1 and C-6 of glucose are then released to CO_2 in the steady state, whereas generation of a C-1 – C-6 difference in CO_2 depends upon deviation of some of the C-6 to other products, such as

Fig. 3. Comparison of the estimated fraction of glucose metabolized to CO_2 and glyceraldehyde-P by the pentose phosphate pathway (broken lines) with the true fraction (solid lines) in a model tissue

Details are as for Fig. 2, except for the change in ordinate variable.

various tissue constituents or lactate released to the medium. Detection of the pentose phosphate pathway in the absence of such deviation would require examination of the time course of the CO_2 outputs during the transient phase after introducing the labelled glucose before the steady state is reached [12], to which the equations considered here are not applicable. Such a situation apparently exists when dorsal root ganglia of chickens are incubated in small volumes of media [12].

One simple alternative approach is to use the difference between the $^{14}\text{CO}_2$ outputs from $[1-^{14}\text{C}]$ glucose and $[6-^{14}\text{C}]$ glucose. This difference has recently been shown to yield a lower limit for the flux from glucose into the pentose phosphate pathway in gluconeogenic tissues as well as in non-gluconeogenic ones and to be applicable during the transient phase after introducing labelled glucose as well as in the steady state [12,19]. Such use of the CO_2 difference requires only the single assumption that the pentose phosphate pathway provides the only mechanism for increasing the ratio C-1 of glucose to C-6 of glucose in CO_2 , to which there presently seems to be no known exception. An upper limit to the flux is obviously set by the steady-state output of $^{14}\text{CO}_2$ from $[1-^{14}\text{C}]$ glucose. Although such upper and lower limits may seem unsatisfying, they appear to be generally applicable and rigorously accurate. Moreover, the range of uncertainty can be minimized by judicious timing of the CO_2 measurements [12]. The unequivocal limits thus obtained should be useful for many experimental purposes, such as revealing that the contribution of the pentose phosphate pathway is substantial. They also afford criteria by which other procedures that involve less certain assumptions might be tested.

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APPENDIX

Derivation of equations

Definitions

G is the rate of glucose uptake by the tissue in mol/h. R is the rate of Fru-6- P production by the pentose phosphate pathway in mol/h. $F_{\text{ent.}}$ is the fraction of glucose that enters the pentose phosphate pathway. Correspondingly, $1 - F_{\text{ent.}}$ is the fraction of glucose that is metabolized via glycolysis, according to assumption 6 in the main paper. $F_{\text{met.}}$ is the fraction of glucose metabolized to CO_2 and triose- P by the pentose phosphate pathway. The fractions $F_{\text{ent.}}$ and $F_{\text{met.}}$, defined above to describe the distribution of glucose, which occurs via the Glc-6- P pool, also obviously apply to other constituents of the latter pool, including the Glc-6- P derived from Fru-6- P formed by the pentose phosphate pathway. N is the fraction of C-3 of glyceraldehyde- P released to CO_2 by the citric acid cycle. C-3 is specified because carbons 1 and 6 of glucose reach only this position in glyceraldehyde- P , next to the phosphate group [1]. $[1]\text{CO}_2$ is the rate of release of C-1 of glucose to CO_2 in mol/h, i.e. the sum of releases by the pentose phosphate pathway and the citric acid pathway. $[6]\text{CO}_2$ is the rate of release of C-6 of glucose to CO_2 in mol/h. This release occurs only via the citric acid cycle in the non-gluconeogenic tissues being considered here (assumption 4a).

Equations for homogeneous tissues

Since the tissue is assumed to be homogeneous (assumption 1) only single values need be used for variables involved in this formulation.

Fraction of glucose entering the pentose phosphate pathway.

Since the tissue is in a metabolic steady state (assumption 2), the rate of entry into Glc-6- P must equal the rate of departure from Glc-6- P . For C-1 of glucose, the only entry into Glc-6- P is directly from glucose itself at rate G . Neglecting glycogen metabolism (assumption 6), the departure of C-1 is divided into (a) a portion which enters the pentose phosphate pathway at rate $G \cdot F_{\text{ent.}}$ and is all released to CO_2 , and (b) a portion that enters glycolysis and goes to position C-3 in glyceraldehyde- P at rate $G \cdot (1 - F_{\text{ent.}})$. Of the latter, a fraction is released to CO_2 by the citric acid pathway at rate $N \cdot G \cdot (1 - F_{\text{ent.}})$.

Whence:

$$[1]\text{CO}_2 = G[F_{\text{ent.}} + (1 - F_{\text{ent.}})N] \quad (\text{A1a})$$

For C-6 of glucose, since none is released to CO_2 by the pentose phosphate pathway and a negligible amount is lost to ribose- P or products of the pathway other than triose- P or Fru-6- P (assumption 3a), all eventually reaches position C-3 in glyceraldehyde- P at rate G (including some that is recycled one or more times through the pentose phosphate pathway). Therefore:

$$[6]\text{CO}_2 = G \cdot N \quad (\text{A1b})$$

Eliminating N between eqns. (A1a) and (A1b) and solving for $F_{\text{ent.}}$ yields:

$$F_{\text{ent.}} = \frac{[1]\text{CO}_2 - [6]\text{CO}_2}{G - [6]\text{CO}_2} \quad (\text{A2a})$$

This can alternatively be written as:

$$F_{\text{ent.}} = \frac{[1]\text{CO}_2/G - [6]\text{CO}_2/G}{1 - [6]\text{CO}_2/G} \quad (\text{A2b})$$

Terms in the form $[x]\text{CO}_2/G$, which represent the fraction of

carbon x of glucose released to CO_2 by the tissue, were called specific yields of CO_2 by Katz & Wood [2] and Wood *et al.* [3].

Fraction of glucose metabolized to CO_2 and triose- P by the pentose phosphate pathway. The flux from glucose into the pentose phosphate pathway is by definition $G \cdot F_{\text{ent.}}$. Due to recycling, two-thirds of the carbon of this glucose reappears in the hexose- P pool as Fru-6- P , while the remaining one-third is metabolized to CO_2 and triose- P (assumption 3). A fraction $F_{\text{ent.}}$ of the Fru-6- P produced by the pentose phosphate pathway is then recycled into this pathway, producing more CO_2 and triose- P as well as additional Fru-6- P , some of which in its turn is recycled. The total amount that will be metabolized over an infinite number of pathways can be found as follows, by considering some total fluxes in the steady state.

First, consider the Fru-6- P produced by the pentose phosphate pathway. According to assumption 6, the total entry into the hexose- P pool (which includes Glc-6- P + Fru-6- P according to assumptions 4b and 4c) is $G + R$, where R is the rate of production of Fru-6- P by the pentose phosphate pathway. Of this hexose- P , a fraction ($F_{\text{ent.}}$) enters the pentose phosphate pathway at rate $(G + R) \cdot F_{\text{ent.}}$. According to the known stoichiometry of the pentose phosphate pathway (assumption 3c), $R = \text{two-thirds of the entry rate}$, so that $R = \frac{2}{3}(G + R) \cdot F_{\text{ent.}}$. Solving the last equation for R yields:

$$R = 2G \cdot F_{\text{ent.}} / (3 - 2F_{\text{ent.}}) \quad (\text{A3a})$$

Next, consider the formation of CO_2 and glyceraldehyde- P by the pentose phosphate pathway. The formation of each of these two products accounts for one-sixth of the carbon in the entering Glc-6- P (assumptions 3b and 3c), so that their sum accounts for one-third of the entering Glc-6- P , or $\frac{1}{3}(G + R) \cdot F_{\text{ent.}}$. By definition, this sum is the amount of glucose metabolized by the pathway. Therefore, the fraction metabolized is given by

$$F_{\text{met.}} = \frac{1}{3}(G + R) \cdot F_{\text{ent.}} / G \quad (\text{A3b})$$

If R in eqn. (A3b) is replaced by R of eqn. (A3a) and the result is solved for $F_{\text{met.}}$, we get:

$$F_{\text{met.}} = F_{\text{ent.}} / (3 - 2F_{\text{ent.}}) \quad (\text{A4a})$$

Solving this for $F_{\text{ent.}}$ gives:

$$F_{\text{ent.}} = 3F_{\text{met.}} / (1 + 2F_{\text{ent.}}) \quad (\text{A4b})$$

Substituting this into eqn. (A2b) gives:

$$\frac{[1]\text{CO}_2/G - [6]\text{CO}_2/G}{1 - [6]\text{CO}_2/G} = \frac{3F_{\text{met.}}}{1 + 2F_{\text{met.}}} \quad (\text{A4c})$$

This is identical with eqn. (1), as derived by Katz & Wood [2] and Wood *et al.* [3] and recently reaffirmed by Landau [4].

Equations for an illustrative non-homogeneous tissue

Here we consider a hypothetical tissue containing two classes of cells, as defined in the introduction to the section entitled 'Formulations for an illustrative non-homogeneous tissue'. Since both classes are assumed to be homogeneous within themselves and to fulfil all assumptions used in developing the equations for homogeneous tissues, those equations can be used to calculate the contributions of the individual classes.

Fraction of glucose entering the pentose phosphate pathway. According to eqn. (A1a):

$$([1]\text{CO}_2)_a = G_a W_a [F_{\text{ent.,a}} + (1 - F_{\text{ent.,a}})N_a]$$

and

$$([1]\text{CO}_2)_b = G_b W_b [F_{\text{ent.,b}} + (1 - F_{\text{ent.,b}})N_b]$$

Adding these two outputs together to get the total [1]CO₂ output for the whole tissue gives:

$$[1]CO_2 = G_a W_a [F_{ent.,a} + (1 - F_{ent.,a}) N_a] + G_b W_b [F_{ent.,b} + (1 - F_{ent.,b}) N_b] \quad (A5a)$$

Correspondingly, from eqn. (A1b), the total [6]CO₂ output is:

$$[6]CO_2 = G_a W_a N_a + G_b W_b N_b \quad (A5b)$$

Obviously, the total glucose uptake is:

$$G = G_a W_a + G_b W_b \quad (A5c)$$

The tissue totals given by the eqns. (A5a)–(A5c) are the values that would be observed experimentally. They accordingly can be substituted into eqns. (A2a) or (A2b) to obtain an 'estimated' value for the fraction of the glucose entering the pentose

phosphate pathway ($F_{ent.,est.}$) that would be calculated from the measurements when ignoring the non-homogeneity of the tissue. The resulting lengthy equation can be reduced by simple algebraic manipulations to the following form:

$$F_{ent.,est.} = \frac{G_a W_a F_{ent.,a} (1 - N_a) + G_b W_b F_{ent.,b} (1 - N_b)}{G_a W_a (1 - N_a) + G_b W_b (1 - N_b)} \quad (A6)$$

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