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Use of calorespirometric ratios, heat per CO_2 and heat per O_2, to quantify metabolic paths and energetics of growing cells☆

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Abstract

The two calorespirometric ratios, the ratio of metabolic heat rate to the rate of CO_2 production and the ratio of metabolic heat rate to the rate of O_2 uptake (R_q/R_{CO_2} and R_q/R_{O_2}, respectively), provide different information about the activities of metabolic pathways. In a steady state system, R_q/R_{CO_2} depends only on the oxidation state of the substrate and R_q/R_{O_2} equals Thornton's constant or the oxycaloric equivalent. In a growing or developing system, the measured ratio R_q/R_{O_2} differs from the oxycaloric equivalent only if reactions that do not consume oxygen and have nonzero ΔH are present. Relative rates of aerobic and anaerobic (with $\Delta H \neq 0$) reactions can thus be calculated from the measured R_q/R_{O_2}, but the substrate carbon conversion efficiency cannot. The difference between the R_q/R_{CO_2} ratio predicted from Thornton's rule and the actual measured ratio contains information on the rates of anaerobic reactions with $\Delta H \approx 0$. The latter ratio thus allows partitioning of the CO_2 production rate between oxidative catabolism and anabolic reactions with $\Delta H \approx 0$. This partitioning allows calculation of substrate carbon conversion efficiency and rates of growth or development.

Keywords: Calorespirometric ratios, Calorimetry, Oxycaloric ratio, Thornton's rule, Respiration, Efficiency, Catabolism, Anabolism

1. Introduction

Understanding and measurement of metabolic efficiency is important for at least two reasons. First is the practical problem of maximizing yield of a desired product from biological cultures as widely varied as fermentation vats and farmers' fields. Second is the fundamental problem of understanding the thermodynamic relations describing metabolism and growth. Three readily measurable properties of respiration in living tissues are the rate of heat production (R_q), the rate of oxygen consumption (R_{O_2}), and the rate of carbon dioxide production (R_{CO_2}). Not considering the inverses as different ratios, three ratios can be formed from these three measures of respiratory rate, i.e. R_q/R_{CO_2}, R_q/R_{O_2}, and R_{CO_2}/R_{O_2} The first two are the calorespirometric ratios discussed here. The last is the respiratory quotient, which has been discussed extensively in treatises on respiratory metabolism, but usually with the assumption of a steady state system. This paper discusses the relations between metabolic efficiency, metabolic paths, and measured values of the calorespirometric ratios in both steady state and in growing or developing systems. There are differences in the information content of R_q/R_{CO_2} and R_q/R_{O_2} so that each ratio provides unique information on metabolic activities and efficiency. Measurement of both ratios provides information not available from either alone.

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Table 1
Typical values of the variables in models used to interpret calorespirometric ratios

Variable	Typical value	Definition
ΔH_{O_2}	-455 ± 15 kJ mol^{-1} O_2	Heat of combustion of organic compounds, Thornton's constant, oxycaloric equivalent
γ_S	0 for carbohydrates	Oxidation number of carbon in substrate
	-1.0 for proteins	
	-1.8 for lipids	
ΔH_{CO_2}	-470 kJ mol^{-1} CO_2 for sugars in aqueous solution	Heat of combustion of organic compounds
	-570 kJ mol^{-1} CO_2 for proteins	
	-660 kJ mol^{-1} CO_2 for lipids	
γ_B	-0.25	Oxidation number of carbon in anabolic products
ΔH_A	0 kJ Cmole^{-1}	Heat of disproportionation of organic compounds
ΔH_B	+10 to +100 kJ Cmole^{-1}	Heat of reduction of organic compounds by removal of oxygen
ε	0–0.9	Substrate carbon conversion efficiency
R_q/R_{O_2}	455 kJ mol^{-1} O_2	Calorespirometric ratio for oxygen
R_q/R_{CO_2}	0–600 kJ mol^{-1} CO_2	Calorespirometric ratio for carbon dioxide

Thornton's rule plays a prominent role in the interpretation of calorespirometric ratios. Thornton's rule states that the enthalpy of combustion of organic compounds is approximately constant when expressed per mole of O_2 [1]. This observation was first made by Thornton in 1917 and was based on heats of combustion of hydrocarbons. The rule was later shown to apply to a wide variety of organic compounds and materials by several others [2], often without reference to Thornton, e.g. see reference [3]. Gnaiger and Kemp [4] and others refer to Thornton's constant as the theoretical oxycaloric equivalent, a term widely used in connection with indirect calorimetry. The numerical value of Thornton's constant or the oxycaloric equivalent varies from -430 to -480 kJ mol^{-1} O_2 depending on the class of compound [5]. If the compound class is unknown, we recommend use of the median value of -455 ± 15 kJ mol^{-1} O_2. Assuming heats of solution are negligible, which appears to be generally true [6], few, if any, compounds in their normal state in biological systems fall outside this range. From the oxycaloric equivalent (ΔH_{O_2}) and the chemical oxidation state (γ_S) of the organic compound being combusted, an estimate of the enthalpy change of combustion per mole of CO_2 released (ΔH_{CO_2}) can also be obtained.

$$\Delta H_{CO_2} = \left(1 - \frac{\gamma_S}{4}\right) \Delta H_{O_2} \quad (1)$$

Also note that, as a consequence of Thornton's rule, for any reaction in which O_2 is taken up by an organic substrate, the enthalpy change is approximately equal to -455 kJ mol^{-1} O_2. CO_2 need not be a product of the reaction. This corollary appears to apply to formation of any oxidation product except peroxides.

The calorespirometric ratios measured on live organisms, tissues, and cultures typically deviate from those predicted from Thornton's rule. This deviation contains the information on metabolic pathways that is available from calorespirometric studies. R_q/R_{CO_2} measurements on living tissues during growth and development may vary from near zero to over 600 kJ mol^{-1} (e.g. see [7,8]). R_q/R_{O_2} values as large as nearly 1500 kJ mol^{-1} have been measured [5]. These large variations from Thornton's rule provide quantitative information on relative activities of metabolic pathways and metabolic efficiencies. However, interpreting the calorespirometric ratios requires a biochemical model. The model is connected to the calorespirometric ratios through an enthalpy balance:

$$R_q = \sum R_i \Delta H_i \quad (2)$$

where R_i is the rate of the ith reaction with an enthalpy change ΔH_i and the sum is over all the reactions in the system. To assist the reader in assimilating these models, Table 1 lists typical values of the variables.

2. Steady state systems, with efficiency = 0

In a steady state system, i.e. non-growing, mature, not developing or senescing organism, tissue, or culture where net accumulation and change in composition of biomass is zero, the only reaction is the catabolic reaction, and R_q/R_{CO_2} and R_q/R_{O_2} are related solely by the oxidation state of the

catabolic substrate. Both plants and animals can approximate a steady state system during some stages of their life cycle. As an example, the guinea pigs used by Lavoisier and Laplace in their experiments reported in 1780 [9] were assumed to be in a steady state. Following Lavoisier and Laplace's conclusion that respiration is a slow combustion, but using modern nomenclature, the catabolic reaction can be represented as oxidation of carbon substrate to CO_2 and water:

$$C_{\text{substrate}} + \left(1 - \frac{\gamma_s}{4}\right) O_2 \rightarrow CO_2 + (-\tfrac{1}{2}(\gamma_s))H_2O + \cdots \tag{3}$$

and the enthalpy balance is:

$$R_q = R_{O_2} \Delta H_{O_2} = R_{CO_2} \left(1 - \frac{\gamma_s}{4}\right) \Delta H_{O_2} \tag{4}$$

For this case the respiratory quotient is:

$$\frac{R_{CO_2}}{R_{O_2}} = \frac{1}{1 - \gamma_s/4} \tag{5}$$

That is, the ratio of the rate of CO_2 production to O_2 uptake is equal to the ratio of the coefficients on CO_2 and O_2 in Eq. (1). In the steady state case, the two calorespirometric ratios are then related by:

$$\frac{R_q}{R_{CO_2}} = \left(1 - \frac{\gamma_s}{4}\right) \left(\frac{R_q}{R_{O_2}}\right) = -\left(1 - \frac{\gamma_s}{4}\right) \Delta H_{O_2} \tag{6}$$

The assumption of a steady state system is the basis for indirect calorimetry in which the heat rate is calculated as the product of the oxycaloric equivalent and the rate of oxygen uptake, i.e. Eq. (4). Because no energy is being conserved in a system in a steady state, the efficiency is zero, no net growth or development is occurring, and the relation between R_{CO_2} and R_{O_2} depends only on the oxidation state of the substrate γ_s. As a historical note, all three ratios can be calculated from the data of Lavoisier and Laplace [9]; $R_{O_2}/R_{CO_2} = 1.23$ from which $\gamma_s = -0.92$, $R_q/R_{CO_2} = 367\,\text{kJ mol}^{-1}$, and $R_q/R_{O_2} = 298\,\text{kJ mol}^{-1}$. These data are the first and one of very few examples where data were collected from which all three ratios can be calculated. Since $R_q/R_{O_2} \neq \Delta H_{O_2}$, the data also show that the guinea pig was not in a steady state as assumed. The assumption of steady state was and is probably incorrect in most indirect calorimetry studies.

3. Growing and developing systems, with efficiency > 0

In growing or developing systems, new cells are being produced and/or their size and composition change with time. For systems in which the substrate is more oxidized than the biomass produced, the overall chemical equation becomes:

$$C_{\text{substrate}} + xO_2 + (\text{compounds of N, P, K, etc.}) \rightarrow \varepsilon C_{\text{bio}} + (1 - \varepsilon)CO_2 \tag{7}$$

where ε is the substrate carbon conversion efficiency or the fraction of substrate carbon retained in anabolic products, i.e. in new cellular material represented here by C_{bio}. This reaction may be conceptually divided into two parts. One being the overall reaction of catabolism, i.e. the oxidative metabolism of $C_{\text{substrate}}$ to CO_2 and water. The other part is anabolism, i.e. all the reactions required for construction of C_{bio} or, in other words, for the construction of the components of functioning cells. With this definition, the rate of production of new cellular material by anabolism is given by:

$$R_{\text{anabolism}} = R_{CO_2} \left(\frac{\varepsilon}{1 - \varepsilon}\right) \tag{8}$$

where $\varepsilon/(1 - \varepsilon)$ is simply the ratio of the coefficients in Eq. (7).

From the enthalpy balance, the rate of heat production during growth and development is given by the rates of the catabolic and anabolic reactions multiplied by their respective enthalpy changes. The rate of heat production can be expressed as a function of the rate of CO_2 production:

$$R_q = -\Delta H_{CO_2} R_{CO_2} - \Delta H_B R_{\text{anabolism}} \tag{9}$$

where ΔH_{CO_2} is the enthalpy change per mole of CO_2 for reaction (3) and ΔH_B is the enthalpy change per Cmole for the reaction:

$$C_{\text{substrate}} + (\text{compounds of N, P, K, etc.}) \rightarrow C_{\text{bio}} + (\tfrac{1}{4}(-\gamma_B))O_2 \tag{10}$$

where γ_B is the average chemical oxidation state of carbon in C_{bio}, the products of anabolism. The rate of heat production can also be expressed as a function of the rate of oxygen consumption:

$$R_q = -\Delta H_{O_2} R_{O_2} - \Delta H_A R_{\text{anabolism}} \tag{11}$$

where ΔH_A is the enthalpy change per Cmole for the reaction.

$$C_{\text{substrate}} + (\text{compounds of N, P, K, etc.}) \rightarrow \left[\frac{4}{\gamma_s - \gamma_B + 4}\right] C_{\text{bio}} + \left[\frac{\gamma_s - \gamma_B}{\gamma_s - \gamma_B + 4}\right] CO_2 \tag{12}$$

Note that the stoichiometries in Eqs. (7), (10), and (12) depend on the oxidation state of nitrogen in the substrate. The stoichiometry of Eq. (7), i.e. ε, additionally depends on growth conditions. In this paper we assume the substrate nitrogen to be fully reduced so no reducing equivalents are used to incorporate nitrogen into the biomass. The quantities of other required elements that may undergo changes in oxidation states are typically too small to be of significance in the overall energy considerations. Also note that intermediates such as ATP and NADH are not included in the reactions because their concentrations, even in growing or developing tissues, remain essentially constant, i.e. in a steady state.

Combining Eqs. (1), (8), and (9) gives:

$$\frac{R_q}{R_{CO_2}} = -\left(1 - \frac{\gamma_s}{4}\right)\Delta H_{O_2} - \Delta H_B\left(\frac{\varepsilon}{1-\varepsilon}\right) \quad (13)$$

which shows how R_q/R_{CO_2} is related to ε. Combining Eqs. (8) and (11) and the respiratory quotient from Eq. (5) gives:

$$\frac{R_q}{R_{O_2}} = -\Delta H_{O_2} - \Delta H_A\left(\frac{\varepsilon}{1-\varepsilon}\right) \times \left\{\left(1 - \frac{\gamma_s}{4}\right) + \left(\frac{\varepsilon}{1-\varepsilon}\right)\left(\frac{\gamma_B - \gamma_s}{4}\right)\right\}^{-1} \quad (14)$$

which shows how R_q/R_{O_2} is related to ε.

4. Interpretation of calorespirometric ratios in terms of efficiencies

Because the oxidation state of carbon in biological tissues, i.e. γ_B, is typically <0 [2] and ΔH_B is typically >0 [6], Eq. (13) can be used to obtain information on ε from measurement of R_q/R_{CO_2}. Determination of ε with Eq. (13) is experimentally challenging however, because ΔH_B is typically only 10–20% of the value of ΔH_{O_2} [3,6,10]. Nonetheless, application of Eq. (13) to calorespirometric data [11,12] produces values of ε similar to those of other methods [3,6,13,14], but with much less effort. Values for tissues from several species of plants show ε varies from 0 to a maximum of about 0.85 $\pm$ 0.05 depending on conditions and age of the tissue [15].

In contrast, Eq. (14) and the ratio R_q/R_{O_2} is impractical for obtaining information on ε because ΔH_A is approximately zero, i.e. by application of Thornton's rule to Eq. (12). The second term in Eq. (14) is therefore negligible and R_q/R_{O_2} is simply equal to the oxycaloric equivalent. Any deviation from this value indicates that reaction (7) is not a complete description of the overall chemistry of the system and other types of reactions with nonzero ΔH must be present. Because of this dependence on other reactions with nonzero heat, the R_q/R_{O_2} ratio is useful in some cases for determining the efficiency of production of byproducts of metabolism [5]. However, the R_q/R_{O_2} ratio is blind to reactions with $\Delta H \approx 0$. For example, when a significant amount of CO_2 is produced by disproportionation of carbon compounds, no O_2 is consumed in the reaction, little heat is produced or consumed by the reaction, and the measured value of R_q/R_{O_2} differs little from the oxycaloric equivalent.

ΔH_A and ΔH_B are often confused in discussions of energy transfer from catabolism to anabolism and thus of energy efficiency. For example, Dejean et al. [16] incorrectly concluded that variation of R_q/R_{O_2} indicated growth efficiency of yeast on lactate substrate because O_2 was included in the anabolic reaction and thus ΔH_A was calculated incorrectly. They estimated ΔH_A as +156 kJ mol^{-1} lactate whereas Battley [17] calculated ΔH_A of −31 kJ mol^{-1} lactate for growth of *Pseudomonas saccharophilia* on lactate. Using the methods of Battley [17,18] and the elemental formula given by Dejean et al. [16], we calculate ΔH_A of −28 kJ mol^{-1} lactate for growth of yeast on lactate. This value for ΔH_A is not only much smaller than that obtained by Dejean et al. [16] but also differs in sign. We have committed the same error, i.e. using R_q/R_{O_2} as a general indicator of efficiency, in some past discussions, but hopefully not in print. The idea that "the less heat produced per oxygen consumed indicates a more efficient system for producing biomass" is incorrect. R_q/R_{O_2} values less than the oxycaloric equivalent instead can only indicate the presence of endothermic reactions which may or may not be beneficial to the organism or in producing a wanted byproduct. The use of the R_q/R_{O_2} ratio to indicate efficiency implicitly assumes reaction (10) describes the actual anabolic reaction in the system, whereas this reaction is only a convenient tool for unraveling the thermodynamics of the system. In contrast, reaction (12) describes the actual anabolic reaction of respiration in most biological systems, and, as a consequence, measured R_q/R_{CO_2} values that are less than the value predicted by Thornton's rule are a quantitative indicator of the efficiency of conversion of substrate into growth.

5. Interpretation of calorespirometric ratios in terms of metabolic paths

A model of the biochemistry of the system is required to obtain information on the relative activities of metabolic paths from calorespirometric ratios. Eq. (3) is an example of such a model for steady state systems and Eqs. (7) and (10) or Eqs. (7) and (12) constitute models for growing or developing systems. The basis for obtaining the relation between the calorespirometric ratios and these models is an enthalpy balance, Eq. (2). Eqs. (9) and (11) are examples of enthalpy balance equations based on the models in Eqs. (7) and (10) or Eqs. (7) and (12), respectively. Because of the obvious direct connection to the oxycaloric equivalent, this approach has most commonly been used with the R_q/R_{O_2} ratio.

R_q/R_{O_2} is the ratio commonly measured on mammalian cells in culture, on aquatic animals, and on microorganisms because R_{O_2} is readily measured electrochemically in aqueous solutions (e.g. see references [5,19]) while R_{CO_2} is more difficult to measure in an aqueous milieu. The general enthalpy balance equation used to interpret measured values of the R_q/R_{O_2} ratio is:

$$R_q = -R_{O_2}\Delta H_{O_2} - \sum R_i \Delta H_i = R_{O_2}(455\,\text{kJ mol}^{-1}) - \sum R_i \Delta H_i \quad (15)$$

and the ratio is given by:

$$\frac{R_q}{R_{O_2}} = 455\,\text{kJ mol}^{-1} - \left(\frac{1}{R_{O_2}}\right)\sum R_i \Delta H_i \quad (16)$$

The R_q/R_{O_2} ratio thus can only deviate from the oxycaloric equivalent if anaerobic reactions with $\Delta H \neq 0$ exist in the

system. Mammalian cells typically have R_q/R_{O_2} values more exothermic than $-455\,kJ\,mol^{-1}$. Because the measured ratio differs from the oxycaloric equivalent it contains quantitative information about the metabolic pathways causing this difference. Kemp [5] models this difference with anaerobic reactions typical in mammalian cells, and shows how such anaerobic reactions cause R_q/R_{O_2} ratios to be larger than $455\,kJ\,mol^{-1}$. These reactions occur normally in fully aerobic systems and are not usually due to anoxic conditions. With knowledge of the chemical nature of the products, calorespirometric data thus enable calculation of the relative amounts of carbon substrate flow through oxidative respiration or synthesis of the product [5]. Because Eq. (16) partitions the reactions according to their relative heat effect, the R_q/R_{O_2} ratio is blind to reactions with $\Delta H \approx 0$. For example, when a significant amount of CO_2 is produced by disproportionation of carbon compounds, no deviation from Thornton's rule is seen in the R_q/R_{O_2} ratio, but deviation does occur in the R_q/R_{CO_2} ratio.

The ratio most commonly measured on terrestrial plants and animals is R_q/R_{CO_2} One model for interpreting deviations of R_q/R_{CO_2} from the value predicted with Thornton's rule was given above in Eq. (13), i.e. in terms of ε. Eq. (9) can also be used to predict growth or anabolic rates from measured heat and CO_2 rates. Note that Eq. (11) cannot generally be used to obtain anabolic rates because $\Delta H_A \approx 0$. Interpreting the R_q/R_{CO_2} ratio with Eqs. (8) and (9) works well for plant tissues under normal growth conditions [20]. However, this model is not unique.

The general enthalpy balance equation for interpreting the R_q/R_{CO_2} ratio is:

$$R_q = -R_{CO_2}\Delta H_{CO_2} - \sum R_i \Delta H_i \tag{17}$$

where ΔH_{CO_2} is now defined differently than in Eq. (9). In Eq. (9), ΔH_{CO_2} is the weighted average of the ΔH values for all the CO_2 producing reactions, of which there are only two types in biological systems. CO_2 can be produced by oxidation of organic compounds by oxygen with $\Delta H = (1 - \gamma_s/4)(-455\,kJ\,mol^{-1})$ or it can be produced by disproportionation reactions with $\Delta H \approx 0$ that produce CO_2 and a more reduced compound. A reasonable approach is thus to partition the CO_2 between these two types of reactions. The calorespirometric ratio is then given by:

$$\frac{R_q}{R_{CO_2}} = f\left(\frac{1-\gamma_s}{4}\right)(455\,kJ\,mol^{-1}) - \left[\frac{1-f}{R_{CO_2}}\right]\sum R_i \Delta H_i \tag{18}$$

where f is the fraction of CO_2 produced by oxygen consuming reactions. This model, which is more generally applicable than the model in Eqs. (7) and (10), partitions the CO_2 rate between aerobic and anaerobic metabolic paths. However, these models are all limited to systems in which all oxygen consumption leads to CO_2. As an example of this aerobic/anaerobic model, deviations from Thornton's rule in the ratio R_q/R_{CO_2} can be analyzed by viewing Eq. (3) as the oxidation or aerobic reaction and Eq. (12) as the disproportionation or anaerobic reaction. While other oxidation (i.e. oxygen consuming) reactions are possible, and need not lead to CO_2 as a product, these are often insignificant in metabolism during growth of terrestrial organisms. Reactions (3) and (12) thus represent actual processes in many organisms. The enthalpy balance in terms of the rate of CO_2 production is:

$$R_q = R_{CO_2}[-f\Delta H_3 - (1-f)\Delta H_{12}] \tag{19}$$

where f is the fraction of CO_2 produced by reaction (3) and $(1 - f)$ is the fraction produced by reaction (12). The enthalpy change for reaction (3), ΔH_3, is given by Thornton's rule as:

$$\Delta H_3 = \left(1 - \frac{\gamma_s}{4}\right)\Delta H_{O_2} = \left(1 - \frac{\gamma_s}{4}\right)(-455 \pm 15)\,kJ\,mol^{-1} \tag{20}$$

Thornton's rule also gives:

$$\Delta H_{12} \approx 0\,kJ\,mol^{-1} \tag{21}$$

Therefore,

$$f \approx \frac{R_q/R_{CO_2}}{455\,(1-\gamma_s/4)} \tag{22}$$

In a completely anaerobic system described by Eq. (12), i.e. in a system in which the substrate is more oxidized than the product biomass, the heat rate is approximately zero, the CO_2 production rate is finite, and therefore $R_q/R_{CO_2} \approx 0$ and $f = 0$. In a completely aerobic system with no net anabolism:

$$\frac{R_q}{R_{CO_2}} = -\left(1 - \frac{\gamma_s}{4}\right)\Delta H_{O_2} = 455\left(1 - \frac{\gamma_s}{4}\right) \tag{23}$$

and $f = 1$. This aerobic/anaerobic model appears to apply to insects in which respiration varies from $f \approx 0$ at low temperatures to $f \approx 0.5$ at higher temperatures [8].

6. Limitations on information obtained on biological systems from only one calorespirometric ratio

Validation of Kemp's model based on R_q/R_{O_2} for mammalian cell cultures [5] required extensive chemical analyses of substrates and products throughout the course of growth of the culture. Simultaneous determination of the R_q/R_{CO_2} ratio prior to the chemical analyses would have shown there was another reaction(s) to be accounted for in the enthalpy balance, allowed calculation of the rate of this (or these) anaerobic reaction and the average oxidation state of the products, and provided an estimate of the enthalpy change for these reactions.

Interpretation of R_q/R_{CO_2} data has problems similar to interpretations of the R_q/R_{O_2} ratio, i.e. chemical information must be determined or assumptions must be made. For

example, at favorable conditions for aerobic growth of terrestrial ectotherms, e.g. plants and insects, the R_q/R_{CO_2} ratio is typically less exothermic than the oxycaloric equivalent [7,11,12]. However, depending on the tissue and the conditions, R_q/R_{CO_2} can be more exothermic than the oxycaloric equivalent, typically when conditions are outside the normal range of growth conditions [7]. Eqs. (13) and (22) can be used to analyze these results. Oxidation of long chain fatty acids can account for R_q/R_{CO_2} values up to about 650 kJ mol^{-1}. Since it is unlikely that fatty acids will be the sole substrate, values of R_q/R_{CO_2} greater than about 500 kJ mol^{-1} likely imply incomplete oxidation of substrate. Simultaneous measurement of R_q/R_{CO_2} and R_q/R_{O_2} could be used to decide between these cases.

Since it would be unusual for γ_s to be positive (i.e. for substrate to be more oxidized than carbohydrate) in these systems, Eq. (13) suggests R_q/R_{CO_2} values less exothermic than the oxycaloric equivalent are due to ΔH_B and ε both being greater than zero. The ratio R_q/R_{CO_2} thus becomes less exothermic as ΔH_B and ε become larger. In immature, rapidly growing, vegetative plant tissues, the substrate is commonly carbohydrate with $\gamma_s = 0$, ΔH_B is typically 25–50 kJ mol^{-1}, and ε ranges from about 0.5 to 0.85 giving $200 < R_q/R_{CO_2} < 430$ kJ mol^{-1}. Values of $R_q/R_{CO_2} < 455$ kJ mol^{-1} are thus taken to be a quantitative indicator of how favorable conditions are for growth or development. Both Eqs. (13) and (22) indicate that R_q/R_{CO_2} can only be more exothermic than the oxycaloric equivalent if γ_s is negative or if aerobic processes are not culminating in CO_2. Because these processes typically occur to a significant extent only when the organism or tissue is stressed or fully mature, $R_q/R_{CO_2} > 455$ kJ mol^{-1} in growing tissues is taken as an indication that conditions are outside the range that can be tolerated for any extended time period. Values of $R_q/R_{CO_2} > 455$ kJ mol^{-1}, which are commonly seen at high and chilling temperatures [7], and in mature plant leaf tissue (unpublished data this laboratory), indicate $\gamma_s < 0$ and/or incomplete oxidation of substrate to CO_2 in oxidative catabolism. Simultaneous measurement of R_q/R_{CO_2} and R_q/R_{O_2} could be used to eliminate the need to make these assumptions about γ_s.

Values of R_q/R_{CO_2} less than about 200 kJ mol^{-1} clearly imply the presence of anaerobic respiration. If the Kreb's cycle is fully active, values of f cannot be less than about 0.38. This calculation is based on minimizing f by the maximum values of ε and ΔH_B (0.85 and 50 kJ mol^{-1}) commonly observed and setting $\gamma_s = 0$ (i.e. carbohydrate substrate). This gives $f = 0.38$, corresponding to $R_q/R_{CO_2} = 173$ kJ mol^{-1}. Combining Eqs. (13) and (22) shows why this is so.

$$f = 1 - \left(\frac{\varepsilon}{1-\varepsilon}\right)\frac{\Delta H_B}{(1-\gamma_s/4)(455)} \quad (24)$$

Simultaneous measurement of R_{O_2} would measure the extent of CO_2 production from O_2 consuming reactions and thus could be used to verify the presence of anaerobic respiration.

7. Information available from both calorespirometric ratios

Using both calorespirometric ratios allows testing of the assumptions required when using models to interpret a single calorespirometric ratio and shows whether assumptions are valid. For example, measurement of R_q/R_{O_2} in addition to R_q/R_{CO_2} allows determination of whether there is significant accumulation of partially oxidized reaction products. Thus, the R_q/R_{O_2} ratio determines whether there is a need to assume that all oxygen consuming reactions lead to CO_2, i.e. whether the $\sum R_i \Delta H_i$ term in Eq. (17) is significant. When this term is significant, values can be obtained to estimate the extent of accumulation of other oxidation products. Measurement of R_q/R_{CO_2} in addition to R_q/R_{O_2} allows determination of whether there is significant anaerobic production of CO_2 and therefore eliminates the blindness of the R_q/R_{O_2} ratio to reactions with $\Delta H \approx 0$. In summary, measurements of both ratios provide important additional information and allow determination of whether assumptions made in a model are valid.

8. Conclusions

The two calorespirometric ratios both yield useful, but different, information about metabolism in growing and developing systems. R_q/R_{CO_2} measurements can provide useful information about substrate carbon use efficiency, but R_q/R_{O_2} usually does not. Both ratios provide information about metabolic pathways, but additional chemical information on metabolic products is often needed to quantify and interpret the results from measurement of a single ratio. Simultaneous measurements of both calorespirometric ratios would eliminate many of the limitations on interpretations of calorespirometric data.

A consequence of fundamental importance following from the measured values of calorespirometric ratios is that, in systems where reaction (12) accurately describes the overall anabolic process, and thus $\Delta H_A \approx 0$, none of the enthalpy generated in catabolism is conserved in the growth of the biomass, a conclusion also reached by others many years ago ([2,21] and refs. therein). However, it is commonly stated and widely supposed that energy from catabolism (reaction 3) is transferred to the chemical bonds of the products of anabolism. This conclusion is based on (a) the fact that, per Cmole, the heat of combustion of C_{bio} is typically greater than that of $C_{substrate}$ [3,6,13], and (b) the conjecture, popularized by Schrödinger [22], that C_{bio} has unusually low entropy. But, as the stoichiometry of Eq. (12) shows, the enthalpies of combustion of $C_{substrate}$ and C_{bio} should not be compared on the basis of a Cmole of each. Instead, the enthalpy of combustion of a Cmole of $C_{substrate}$ must be compared with the enthalpy of combustion of $[4/(\gamma_s - \gamma_B + 4)]$ Cmole of C_{bio}. The fact that $\Delta H_A \approx 0$ (i.e. ΔH for reaction 12), demonstrates the enthalpies of combustion of the substrate and the

appropriate amount of C_{bio} are the same. Thus, there is essentially no enthalpy from catabolic oxidation of $C_{substrate}$ transferred to the chemical bonds of C_{bio}. The answer to the question of how much Gibbs free energy is transferred from catabolism to anabolic products thus rests solely on the validity of Schrödinger's conjecture, an issue we intend to pursue in a later paper.

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