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CONCEPTS ON EFFICIENCY IN BIOLOGICAL CALORIMETRY AND METABOLIC FLUX CONTROL*

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SUMMARY

Accurate definitions of efficiency are required to resolve controversies on the significance and comparability of measures of efficiency in biological energetics. This review on concepts of efficiency is arranged into 4 parts. First, some fundamental energy relations of equilibrium and nonequilibrium thermodynamics are defined and placed into a coherent context as relevant for efficiency in biology. The classical expression of the Carnot efficiency of a heat engine obtains a new meaning in terms of flux-force relations of nonequilibrium thermodynamics. Second, within this general thermodynamic frame, the specific treatment of energy transformations of chemical reactions is introduced, with particular emphasis on open systems with internal transformation and external transfer of matter. Third, the chemical transformations in ATP turnover and internal efficiencies of coupled reactions are analyzed in two parts. On the one hand, the enthalpy efficiency is relevant in the context of biological calorimetry in relation to uncoupling and the integration of aerobic and anaerobic metabolism. On the other hand, the molar Gibbs energy efficiency relates to the driving force of coupled reactions and to the control of flux. High metabolic power and maximum efficiency are mutually exclusive. Finally, the discussion of various expressions of efficiency in biological growth requires a careful distinction between energy conservation in transformations (chemical reactions) and energy acquisition in coupled transformation and transfer of energy in the form of externally supplied matter. Better understanding and management of biological resource utilization requires this combined analysis of efficiency in biological energetics.

INTRODUCTION

"*La respiration est donc une combustion, à la vérité fort lente*" - *Respiration is therefore a combustion, a very slow one to be precise.* With these remarks, Lavoisier and LaPlace (1783) reflected upon the idea of using calorimetry to explore the *sources* and *dynamics* of metabolic heat changes (ref. 1). Today, methodological advances in biological calorimetry focus both on high accuracy for complete energy balances, and on a high time resolution for the representation of the actual dynamics of heat flux (ref. 2). The relation between enthalpy and heat changes of metabolic reactions is generally accepted (for an exception see ref. 3),

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whereas the relation between thermodynamic functions of state and the dynamics of processes (flows or fluxes) remains controversial. Nonequilibrium thermodynamics postulates a linear relation between metabolic flux and Gibbs force (molar Gibbs energy of reaction) in the near-equilibrium domain. On the other hand, kinetics relates chemical flux to the activities of reactants. A unification of these contrasting views is one of the major challenges of modern theoretical physical chemistry.

Concepts on efficiency of energy and e*x*e*r*gy conversion are dealt with in classical thermodynamics and in nonequilibrium thermodynamics or *erg*odynamics (ref. 1,4). Not surprisingly, therefore, thermodynamic and ergodynamic considerations are prominent in studies of biological energy transformation where the interest lies in the dynamics of the integrated metabolic process of energy coupling in mitochondria, cells and whole animals (ref. 5,6). From the point of view of kinetics, however, the mere definition of an efficiency of ATP production was criticized (ref. 7), and the importance of chemical driving forces and output/input power ratios for the control of metabolic flux has frequently been denied or ignored. A distinction of various aspects of efficiency is required, depending upon the specific context of a study. In an interdisciplinary context it is important to reiterate some fundamental thermodynamic concepts, providing links between quantities which are otherwise treated separately. The integration of experimental calorimetric and biochemical approaches and thermodynamic and kinetic theories provide a fruitful basis for advancing our understanding of biological energetics.

WARMING UP: THE CARNOT EFFICIENCY

For evaluation of concepts on efficiency (ref. 8) it is necessary to provide some general definitions, with particular emphasis on dimensions and units [given in brackets]. Efficiency is always a *dimensionless* output/input ratio. Therefore, work [J] per amount of oxygen or ATP [mol] is not an efficiency. From such ratios the correct efficiency [$J.J^{-1}$] can be calculated by converting oxygen consumption or ATP turnover into energy units with the aid of chemical potentials (ref. 1). Not every dimensionless output/input ratio is an efficiency. For instance, the amount of ATP produced [mol] per unit catabolic substrate utilized [mol] is a dimensionless stoichiometric ratio. In the biochemical literature, this stoichiometric ratio is sometimes referred to as "biochemical efficiency". However, ATP production and substrate utilization must be converted to energy units. Then the ratio of energy units is the efficiency of ATP production. The *SI* units corresponding to physicochemical quantities will be noted repeatedly since this provides clarity of concepts and communication.

The efficiency of a heat engine is defined as the ratio of the work done on the surroundings to the heat input at the higher temperature, T_h [K] (ref. 8). Let $d_{ext}Q_h.dt^{-1}$ be the externally supplied heat flow at T_h into the unit system size (heat flux, a positive value when heat is added). At steady state, the heat added equals the heat leaving internally the high temperature region during the same time interval, $d_{int}Q_h = -d_{ext}Q_h$. The maximum work per unit time and unit volume, $dW_{max}.dt^{-1}$ [W per unit system size], has a negative value when work is performed by the system. Then the maximum Carnot efficiency is $-F_Q$ (ref. 8,9),

$$F_Q = \frac{dW_{max}.dt^{-1}}{-d_{int}Q_h.dt^{-1}} = -\frac{T_h - T_l}{T_h} \qquad (1)$$

where T_l is the absolute temperature of the heat sink. Therefore, the maximum Carnot efficiency is always <1, except for the impossible condition of $T_l = 0$ K. The difference between heat and work in eq.(1) is the heat exchanged with the heat sink at T_l, dQ.

Heat and muscular power

The concept of maximum Carnot efficiency can be applied to theoretical issues of biological energetics: Is it possible for living cells to operate as heat engines, that is to convert the energy stored in a temperature gradient into a form of work? Consider, for example, the power of exercising man. The highest mechanical aerobic power output, ${}_{out}P$, of a healthy non-athlete (70 kg) is -4 $mW.g^{-1}$ which can be performed for a period of several minutes (ref. 10). At a muscle temperature of 40 °C (T_h = 313 K) and an ambient temperature of 20 °C (T_l = 293 K), F_Q is -20/313=-0.064. At the maximum possible Carnot efficiency of 0.064 (6.4%), the metabolic heat necessary to generate -4 $mW.g^{-1}$ of power output can be calculated from Eq.(1) as 4/0.064 = 63 $mW.g^{-1}$. The maximum aerobic metabolic heat flux produced by a top athlete, however, is only 26 $mW.g^{-1}$ (ref. 10). Even at maximum efficiency, therefore, the principle of a heat engine would not have allowed the evolution of powerful biological systems. If the top athlete would operate at a Carnot efficiency of 25% at an ambient temperature of 20 °C, the muscle would have to heat up to 118 °C (391 K).

Fluxes and forces

The dynamics of energy transformations impose an additional constraint on efficiency, since the maximum efficiency can only be attained at the limit of a reversible process. 100% reversibility implies zero flux, when all driving (input, exergonic) forces are completely offset

by compensating (output, endergonic) forces. A generalized flux, J_Y (flow per unit of system size), is measured as the transfer or transformation of a specified quantity Y (heat, charge, extent of reaction, amount of substance, mass; each expressed per unit of system size) per unit of time,

$$J_Y = \frac{dY}{dt} \tag{2}$$

The corresponding generalized force, F_Y, is the partial derivative of potential energy (Gibbs energy, G; expressed per unit of system size) per transmitting quantity, Y,

$$F_Y = \frac{\partial G}{\partial Y} \tag{3}$$

The flux of an external transfer is positive when Y is added to the system, and the flux of an internal transformation of Y is positive when it proceeds in the specified direction of the transformation. Thus internal heat flux from high to low temperature is a positive quantity, ${}_{int}J_Q = -d_{int}Q_h.dt^{-1}$, and $dY = -d_{int}Q_h$ (eq. 2).

For any particular process, force and flux are defined such that their product yields power [W per unit system size], P,

$$P = J_Y F_Y = \frac{dY}{dt}\frac{\partial G}{\partial Y} \tag{4}$$

At a negative value of F_Y (exergonic, eq. 3), flux proceeds spontaneously in the forward direction (then J_Y has a positive value). In the absence of coupling or any other interferences, the power is irreversibly dissipated (negative value of P, eq. 4). According to eq. 4, thermal power is the product of internal heat flux and the corresponding force, F_Q (ref. 9),

$$P = {}_{int}J_Q F_Q = \frac{-d_{int}Q_h}{dt}\frac{\partial G}{-\partial_{int}Q_h} = \frac{dG_Q}{dt} \tag{5}$$

The Gibbs energy of heat transformation, dG_Q, equals the maximum amount of work which can be obtained, dW_{max} (eq. 1). Therefore,

$$\frac{-d_{int}Q_h}{dt} F_Q = \frac{dG_Q}{dt} = \frac{dW_{max}}{dt} \tag{6}$$

Comparison of the force, F_Q, in eq.(6) and the Carnot efficiency, $-F_Q$, in eq.(1) shows that the negative ***Carnot efficiency is identical to the thermal force***. The driving force for heat flux from a heat source to a heat sink is the relative temperature difference,

$$F_Q = \frac{T_l - T_h}{T_h} \tag{7}$$

For any given system, the heat flux is a function of the thermal force (flux/force relation),

$$_{int}J_Q = -L\, F_Q \tag{8}$$

L is the thermal conductivity. With increasingly exergonic thermal force, a given amount of heat is equivalent to an increasing amount of Gibbs energy potentially available for performing work (eq. 6). At zero force, $T_l = T_h$, no exergy is available from any amount of heat exchanged. Isothermal heat flux is a transfer process without energy transformation, at zero thermal power and zero internal entropy production.

In poikilothermic organisms or cultured cells operating at environmental temperature, the calorimetrically measured heat flux, $J_Q = dQ.dt^{-1}$, is nearly isothermal. Therefore, the process of heat conduction from the reaction system to the environmental heat sink, $d_{int}Q_h = dQ$, contributes a small component of the total dissipated exergy (Gibbs energy) of cellular metabolism. Under these conditions, metabolism can be described as an isothermal process, particularly when monitored with virtually isothermal heat flow calorimeters where temperature differences are in the range of 10^{-6} to 10^{-4} K (ref. 11).

In biological energetics, it is best to avoid the term efficiency for $-F_Q$, referring to thermal force instead. Although the discussion of heat engines yields only a negative result, namely that biological systems must operate in a different way (and this is true for the technology in a society aiming at conservation of irreplaceable energy resources), important thermodynamic relations can be obtained which are applicable to energy transformations in general. Substituting in eq.(6) for F_Q from eq.(7) yields,

$$dG_Q = d_{int}Q_h - \frac{T_l}{T_h}\, d_{int}Q_h \tag{9}$$

The internal heat change at constant pressure is accounted for in terms of the system's enthalpy change due to the process of thermal energy transformation, $d_{int}Q_h \equiv dH_Q$ (ref. 6). The second term on the right side of eq.(9) is the amount of heat which cannot be converted into work even at maximum efficiency. It is the unavailable or *bound* energy in the thermal energy transformation, dB_Q,

$$dB_Q = \frac{T_l}{T_h}\, d_{int}Q_h \tag{10}$$

The distinction between Gibbs energy and enthalpy (Fig. 1),

$$dG_Q = dH_Q - dB_Q \tag{11}$$

is of particular importance in chemical reactions.

Energy and exergy

The Carnot "efficiency" is a ratio of work and heat (eq. 1). These energies have different qualities. Strict definitions of efficiency require a correction for this difference in quality (to avoid comparing 'apples with oranges'). Quantities with the dimension of energy [J] are separated into two different groups on the basis of the potential for interconversion, quantified by the (theoretical) maximum efficiency. At maximum efficiency, ***exergy*** (A or G) is fully converted into any other form of work. Therefore, work, W, is an exergy. Helmholtz energy, A, is the exergy including pressure-volume work, and Gibbs energy, G, is the exergy in excess of pressure-volume work. At zero efficiency, ***energy*** (sensu strictu: U or H) is fully converted into heat. Therefore, heat, Q, is an energy. Internal energy, U, is in the absence of work converted into heat under conditions of constant volume; enthalpy, H, under conditions of constant pressure. Measurements of thermal changes are at the heart of ***therm***odynamics. When exergy changes are considered exclusively, irrespective of the thermal changes (particularly in nonequilibrium "thermodynamics"), the term ***erg***odynamics is suggested to emphasize the important distinction between exergy related to work, and energy related to heat (ref. 4).

If an amount of exergy dG over a period of time dt is dissipated at zero efficiency, all the exergy is irreversibly destroyed and consequently has a negative sign. The corresponding *dissipated energy* ($dG \twoheadrightarrow dD$) may be larger or smaller than the isothermal *heat change*, dQ, depending on the nature of the transformation process. In the dissipation of thermal Gibbs energy across a temperature gradient, the heat change at the low temperature heat sink, dQ, is always more negative (exothermic) than the dissipated energy, $dQ \leq dD_Q$ (Fig. 1a; for dB_Q the arrow is pointing downwards). In general, the difference between heat and dissipated energy is the exothermic or endothermic *bound energy,* dB (Fig. 1). The bound energy is the reversible energy change, that is the heat change measured when the process is carried out reversibly ($dG \twoheadleftarrow dW$). For many exergy transformations such as mechanical or electrical, the bound energy is always zero. This is a necessary condition for electrical calibrations of a calorimeter when electrical exergy is irreversibly dissipated across a resistor and is fully converted into heat with zero bound energy, $dG_{el} \twoheadrightarrow dQ$ (irreversible transformation). In general, the heat exchanged with the heat sink and measured calorimetrically is the sum of the "irreversible" or dissipated energy, dD, and the "reversible" or bound energy, dB (Fig. 1),

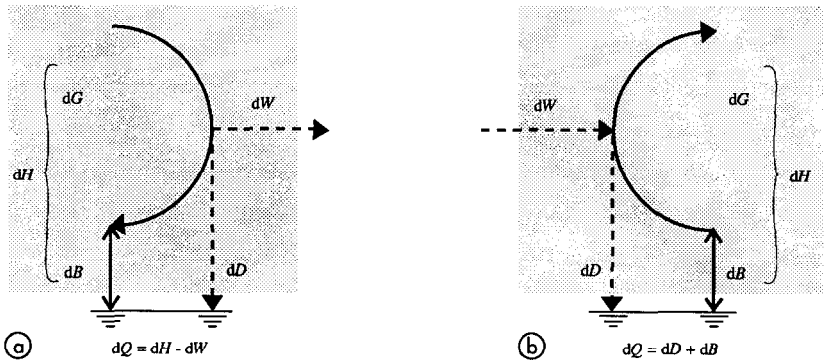

Fig 1. Energy and exergy transformation diagrams. **a**: Exergonic process performing work. **b**: Endergonic process on which work is performed. In both cases the irreversibility is expressed by the dissipated energy, dD, which must be negative. Gibbs energy changes of the transformation, dG, are shown by half cycles, bound energy changes, dB, by straight lines. In contrast to these state functions which are exact differentials, the inexact differentials are indicated by broken lines. Negative values are indicated by half cycle arrows and vertical arrows facing downwards and by horizontal arrows pointing away from the process and the system indicated by the shaded box. Modified after ref. 6.

$$\mathrm{d}Q = \mathrm{d}D + \mathrm{d}B \tag{12}$$

The bound energy is more familiar in the context of eq.(13) (the general form of eq. 11), where the discrepancy between the two state functions energy (enthalpy) and exergy (Gibbs energy) is due to dB (Fig. 1),

$$\mathrm{d}H = \mathrm{d}G + \mathrm{d}B \tag{13}$$

Owing to the important difference between exergy and energy, a terminological distinction is made for the time derivatives of these quantities [$J.s^{-1}$ = W]. Exergy per unit of time is power (eq. 5), whereas energy per unit of time is energy flow (eq. 2; ref. 9). The differential notation dQ, dG, etc. indicates changes per time interval dt. Other cases are stated explicitly.

FROM HEAT ENGINES TO ATP TURNOVER

Chemical flux and extent of reaction

Since cells transform chemical energy (exergy), chemical fluxes and forces and energy changes in chemical reactions are of major interest. Specifically, mitochondrial and total cellular ATP production involves chemical energy transformations

in energetically coupled reactions. A substrate *i* supplied externally to an open system, $d_{ext}n_i$, is expressed in terms of external enthalpy or Gibbs energy transfer ($d_{ext}G_{m,1}$ in Fig. 2). Substrates are internally transformed into products in the process of a reaction, r. At steady state, the amount of substrate added externally during a time interval dt equals the amount of internally reacting substrate, $d_{ext}n_i = -d_r n_i$. These external changes and internal transformations are analogous to $d_{ext}Q_h$ and $d_{int}Q_h$ in the treatment of heat transformation and Carnot efficiency. In the transformations, heat or substrates are removed when the flux proceeds in the forward direction. Therefore, $d_r n_i$ is negative for all consumed substrates but positive for all products generated in the reaction. The internal flux of a reaction, ${}_rJ_B$ [mol B.s^{-1} per unit system size] is calculated according to eq.(2), where the amount of the transmitting quantity, Y, is the extent of reaction, $dY = d\xi$,

$$ {}_rJ_B = \frac{d\xi}{dt} \quad ; \qquad |\nu_B| = 1 \tag{14} $$

The extent of reaction relates the change of substance to the reaction stoichiometry (ref. 12),

$$ d\xi = \frac{d_r n_i}{\nu_i} \tag{15} $$

where ν_i is the stoichiometric number of the *i*th substance, negative for substrates and positive for products. The extent of reaction is the amount of transformed substance independent of choosing a particular substrate or product in a reaction, e.g. 0 = -A -2B +C. However, the extent of reaction depends on the form of the reaction equation, being different for the form 0 = -0.5A -B +0.5C. Therefore, the flux of substance B (eq. 14) implicitly defines the particular stoichiometric form of a reaction.

Chemical power and heat flux

Chemical power, $P = dG_r.dt^{-1}$ (eq. 5) is the product of chemical flux and force (eq. 4),

$$ P = {}_rJ_B \; {}_rF_B \tag{16} $$

According to eq.(3) and Y defined in eq.(14), chemical force is the partial derivative of Gibbs energy per extent of reaction, at constant temperature, pressure and composition,

$$ {}_rF_B = \frac{\partial G}{\partial \xi} \quad ; \qquad |\nu_B| = 1 \tag{17} $$

The chemical force is a partial molar derivative of Gibbs energy [J.mol^{-1} B], commonly known as the negative affinity or molar Gibbs energy of reaction (symbol: $-A = \Delta_r G$; ref. 12). The term ***Gibbs force*** was suggested for ${}_rF_B = \Delta_r G_B$ (ref. 6). The standard terminology

conceals the important difference between Gibbs energy and force.

The Gibbs energy change [J per unit system size] in a reaction system (Fig. 2) equals the sum over all simultaneous reactions, rj,

$$dG_r = \sum_j dG_{rj} \qquad (18)$$

Let the exergonic transformation 1 in Fig. 2 be the catabolic reaction, k, with oxygen flux (eq. 14 and 15) defined as ${}_kJ_{O_2} = -d_k n_{O_2} . dt^{-1}$ [mol $O_2 . s^{-1} . g^{-1}$], and the exergonic transformation 2 be the phosphorylation reaction, p, forming ATP from ADP at a flux ${}_pJ_{ATP}$. Then metabolic power (eq. 16-18) is,

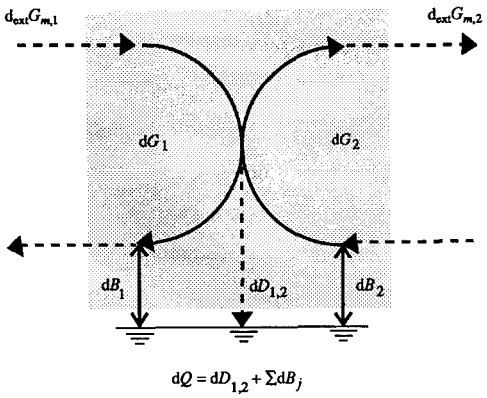

Fig. 2. Energy and exergy transformation diagram of an open system with two coupled reactions, combining the transformations in Fig. 1a and 1b, e.g. mitochondrial ATP production coupled to catabolic substrate oxidation. $d_{ext}G_{m,1}$ and $d_{ext}G_{m,2}$ are the Gibbs energy changes in transfer of substrates and products in reaction 1 and 2.

$$\frac{dG_r}{dt} = {}_kJ_{O_2}\ {}_kF_{O_2} + {}_pJ_{ATP}\ {}_pF_{ATP} \qquad (19)$$

where ${}_kF_{O_2} = \Delta_k G_{O_2}$ is the exergonic Gibbs force of catabolic oxygen consumption [J.mol^{-1} O_2], e.g. eq.(24.1). ${}_pF_{ATP} = \Delta_p G_{ATP}$ is the endergonic Gibbs force of phosphorylation [J.mol^{-1} ATP], eq.(24.2). An equivalent form of eq.(19) yields the enthalpy change, dH_r, replacing the molar Gibbs energies by molar enthalpies of reaction,

$${}_rF_B = \Delta_r G_B = \sum_i \nu_i \mu_i \qquad (20)$$

$$\Delta_r H_B = \sum_i \nu_i H_i = \frac{\partial H}{\partial \xi} \qquad (21)$$

μ_i and H_i are the chemical potential (partial molar Gibbs energy) and the partial molar enthalpy of the ith substance (ref. 12). Gibbs energy and enthalpy have no absolute values and are measured only relative to a specified reference (like using a reference electrode). A commonly used reference state is that of the elements in their most stable state under specified standard conditions. Then Gibbs energies and enthalpies of formation of the ith substance in its actual state are used as a measure of μ_i and H_i in eqs.(20) and (21).

Clarification of the differences and analogies in the thermodynamic description of Gibbs energy and enthalpy changes sets the stage to turn the discussion to the efficiency of a chemical energy transformation of the structure depicted in Fig. 2. It is essential that clear distinctions be made between thermodynamic enthalpy efficiency, and ergodynamic Gibbs energy efficiency. Each is different from the Carnot "efficiency" which is an exergy/energy ratio. *Ergodynamic efficiency*, η_G, is a ratio of *Gibbs energies* of endergonic and exergonic reactions, equivalent to a power ratio (ref. 13). From the input (k) and output process (p) in eq.(19) the ergodynamic efficiency is obtained as a flux ratio times force ratio,

$$\eta_G = -\frac{dG_{r2}}{dG_{r1}} = -\frac{{}_pJ_{ATP}}{{}_kJ_{O_2}} \frac{{}_pF_{ATP}}{{}_kF_{O_2}} \tag{22}$$

Normalization of the flux and force ratios (eq. 22) by the stoichiometry (Fig. 3a) yields the flux efficiency and force efficiency, each with a maximum theoretical value of 1 (ref. 4). A flux efficiency of 1 implies full coupling. *Thermodynamic efficiency*, η_H, is defined in analogy as a ratio of *enthalpies* of endothermic and exothermic reactions,

$$\eta_H = -\frac{dH_{r2}}{dH_{r1}} = -\frac{{}_pJ_{ATP}}{{}_kJ_{O_2}} \frac{\Delta_p H_{ATP}}{\Delta_k H_{O_2}} \tag{23}$$

These relations are applied below in an analysis of the efficiency of ATP production in cellular metabolism, substituting the coupling stoichiometry (Tab. 1) for flux ratios in the case of fully coupled reactions.

THERMODYNAMIC EFFICIENCY, ATP COUPLING, AND CALORIMETRY

The thermodynamic enthalpy-efficiency of net ATP generation is important for interpretations of metabolic heat flux in transient states when net ATP is produced, for instance during recovery from anoxia (ref. 14). Moreover, compartmentalization of cellular metabolism locally separates ATP production from ATP utilization to a large extent. Then the ATP producing compartment (e.g. mitochondria, glycolytic chain) can be defined as the system which exports ATP. A major experimental problem is the accurate quantification of ATP flux under steady state conditions. However, an ATP/O_2 flux ratio of 6.2 is known from studies of muscle tissue under recovery conditions when phosphocreatine accumulates and glycogen is the catabolic substrate (ref. 15), indicative of practically fully coupled mitochondria. The coupled reactions of oxidative phosphorylation (kp) are conventionally written as two separate equations for catabolic *input* (k) and phosphorylation *output* (p), for instance

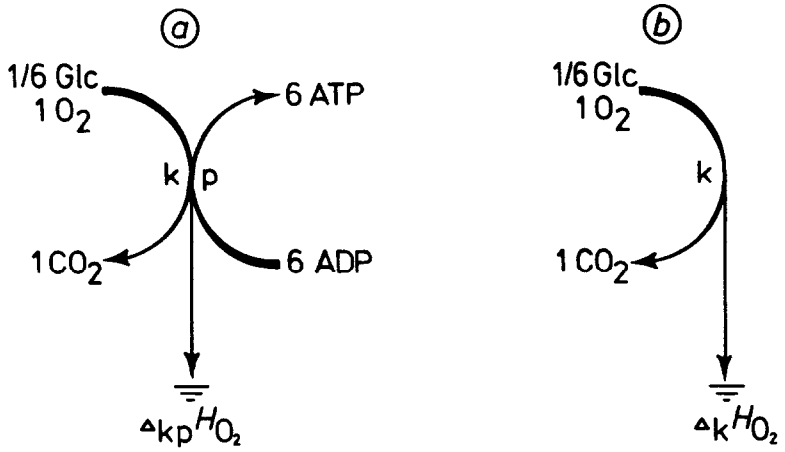

Fig. 3. Energy transformation half cycles in (*a*) fully coupled conservative metabolism, and (*b*) uncoupled catabolism. *a*: Net synthesis of ATP in fully coupled metabolism (thermodynamic efficiency in Tab. 1). The enthalpy change per mol O_2 is the sum of the catabolic (k, exothermic) and phosphorylation (p, endothermic) half cycle (Eq. 24; compare Fig. 2). *b*: The dissipative catabolic half cycle provides the stoichiometric basis for calculating the oxycaloric equivalent, $\Delta_k H_{O_2}$.

for glucose oxidation (Fig. 3a),

$$\text{k:} \quad 0 = \text{-1/6 Glc} - O_2 + CO_2 + H_2O \tag{24.1}$$

$$\text{p:} \quad 0 = 6 \times (\text{ - ADP} - P_i + \text{ATP} + H_2O) \tag{24.2}$$

Such a scheme assumes that, irrespective of mechanism, chemosmotic potentials and intermediary states are maintained at steady state. The corresponding endothermic enthalpy of phosphorylation is $\Delta_p H_{ATP}$ (p, Fig. 3a). The same but negative, exothermic value is the enthalpy of ATP hydrolysis (-p, Fig. 4).

Molar enthalpies of coupled and uncoupled catabolism

The enthalpy of phosphorylation depends on cellular pH, enthalpy of neutralization, and cellular pMg (ref. 16). $\Delta_p H_{ATP}$ is 32 kJ.mol^{-1} at pH 6.5 and increases up to 55 kJ.mol^{-1} towards pH 8 and cellular enthalpies of neutralization (ref. 17). At pH 7.0, magnesium ion activity of pMg 2.5 to 3.5 and intracellular enthalpy of neutralization of -25 kJ.mol^{-1} H^+, $\Delta_p H_{ATP}$ is 43 kJ.mol^{-1} ATP which accounts for both proton and magnesium ion buffering (ref. 17). It must be noted that the enthalpy change is independent of the concentrations of ATP, ADP and inorganic phosphate, in contrast to the Gibbs force.

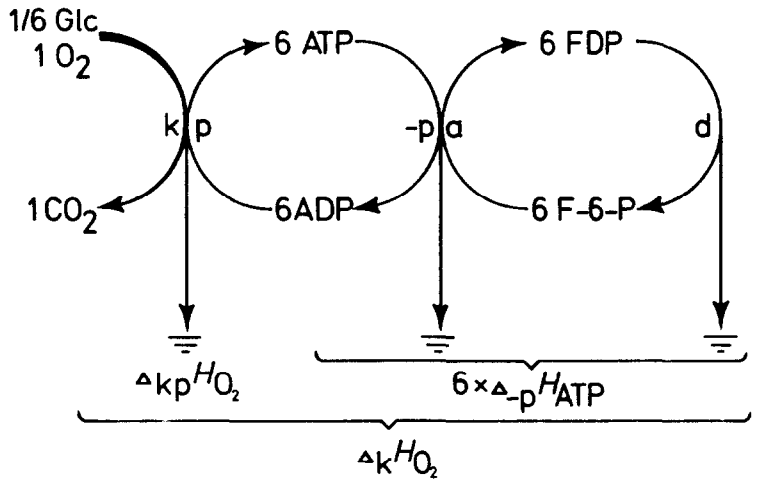

Fig. 4. Half cycles in dissipative maintenance metabolism with steady state ATP turnover, decoupled by futile cycling. The fructose 6-phosphate/fructose 1,6-bisphosphate cycle is shown as an example. The net enthalpy change is calculated from the net biochemical change which, at steady state levels of ATP and all anabolic intermediates, is exclusively due to the catabolic half cycle reaction, equivalent to uncoupled catabolism (oxycaloric equivalent, Fig. 3b). Enthalpy is intermittently conserved in endothermic half cycles (p, phosphorylation; a, anabolic), but an equivalent amount of enthalpy is exothermic in the reversed exergonic half cycles (-p, dephosphorylation; d, dissipative). Therefore, ATP turnover and futile cycling raise the heat flux strictly proportional to the catabolic flux which, however, can be augmented by anaerobic catabolism with a corresponding anaerobic contribution to total heat flux.

An enthalpy of 258 kJ (i.e. 6 x 43) is metabolically conserved in fully coupled net ADP phosphorylation (Eq. 24.2), for each mol of oxygen utilized in the respiration of glucose (Fig. 3a). Therefore, the highest aerobic thermodynamic efficiency, η_H, under cellular conditions stated above is 0.55 (i.e. 6 x 43/469), using the oxycaloric equivalent of -469 kJ.mol^{-1} O_2 for glucose,

$$\eta_H = \frac{\mathrm{ATP/O_2} \times \Delta_p H_{\mathrm{ATP}}}{\Delta_k H_{O_2}} = \frac{\Delta_k H_{O_2} - \Delta_{kp} H_{O_2}}{\Delta_k H_{O_2}} \qquad (25)$$

The enthalpy change per mol O_2 of the fully coupled reaction is (Fig. 3a),

$$\Delta_{kp} H_{O_2} = \Delta_k H_{O_2} + \mathrm{ATP/O_2} \times \Delta_p H_{\mathrm{ATP}}$$
$$\Delta_{kp} H_{O_2} = -469 + 6 \times 43 = -211 \text{ kJ.mol}^{-1}\ O_2,$$

The ATP/O_2 ratio decreases with uncoupling. So the thermodynamic efficiency (eq. 25) is lowered, and the enthalpy change per mol O_2 decreases from -211 to maximally -469 kJ.mol^{-1} for fully uncoupled respiration when CO_2 and O_2 are exchanged with the gas phase (ref. 18).

Calorimetric-respirometric ratios and efficiency

The oxycaloric equivalent, $\Delta_k H_{O_2}$, is the enthalpy of aerobic catabolic reactions per mol O_2 consumed, ranging from -430 to -480 kJ.mol^{-1} O_2 (ref. 1,18). This is the expected ratio of calorimetrically measured heat flux and respirometric oxygen flux (CR ratio [kJ.mol^{-1} O_2]) under conditions when no work is produced and when the reaction system is fully described by the aerobic catabolic half cycle (Fig. 3b). CR ratios determined in various aquatic animals under aerobic conditions (ref. 1) and in fully aerobic microbes (ref. 19) are within the range of theoretical $\Delta_k H_{O_2}$ values. A positive efficiency of net ATP production implies the expectation of a less exothermic CR ratio. Highly exothermic CR ratios up to -1100 kJ.mol^{-1} O_2 (ref. 18) obtained in isolated mammalian cells upon uncoupling or increased futile substrate cycling have been interpreted in terms of a decreased efficiency of ATP production (ref. 20). However, Figs. 3b and 4 illustrate that CR ratios more negative than -480 kJ.mol^{-1} cannot be attributed to low efficiency, since $\Delta_k H_{O_2}$ values are calculated on the assumption of zero net efficiency, irrespective of intermittent efficiencies of ATP production in the phosphorylation reaction (Fig. 4). The observed highly exothermic CR ratios must in fact be explained by the activation of anaerobic metabolism (ref. 18,21).

The enthalpy efficiency of chemical reactions is not subject to the restrictions of the Carnot "efficiency" nor is it necessarily <1. Like other reactions, coupled reactions can be endothermic when proceeding spontaneously. The maximum enthalpy efficiency depends not only on approximately reversible conditions but on the bound energy change. The bound energy change is strongly dependent on the enthalpy of neutralization of cellular or environmental buffers, particularly in anoxic catabolism with the production of strong organic acids. Therefore, efficiencies must be calculated for any particular reaction condition. A reasonable range of efficiencies in fully coupled aerobic and anaerobic catabolism is listed in Table 1, assuming cellular accumulation or excretion of organic acids, at an enthalpy of phosphorylation of 43 kJ.mol^{-1}. The high enthalpy efficiency in propionate-acetate fermentation should be noted (>100%), keeping in mind, however, that particularly in animals under anoxia ATP is never accumulated but rather declines in concentration or is kept at steady state. At a theoretical 100% accumulation or export of ATP to a different compartment, heat would be absorbed from the surroundings on the basis of propionate fermentation.

Table 1. Thermodynamic versus ergodynamic ATP coupling efficiencies in aerobic and anaerobic catabolism of glycogen. Anaerobic pathways are indicated by end products. See ref. 1 and 4 for coupling stoichiometries (ATP/Glycolsyl-unit) and cellular reaction conditions.

	ATP/Glycosyl-unit	Enthalpy	Gibbs energy
Aerobic	37	0.56 - 0.57	0.64 - 0.92
Lactate	3	0.67 - 0.80	0.56 - 0.63
Succinate	4.7	0.78 - 0.87	0.70 - 0.74
Propionate + acetate	6.3	1.02 - 1.21	0.79 - 0.87

On the other hand, thermodynamic efficiencies of aerobic (gross) ATP production in the range of 50 to 55% appear to be very low. This is indeed the case when comparing thermodynamic enthalpy and ergodynamic Gibbs energy efficiencies of aerobic ATP production. However, a much too low thermodynamic enthalpy efficiency of 25% has been reported (ref. 22) due to the neglect of important side reactions and enthalpies of cellular proton buffering (ref. 23).

ERGODYNAMIC ATP EFFICIENCIES: ECONOMY CONTRA POWER

Maximum and optimum efficiency

The ergodynamic or Gibbs energy efficiency of ATP turnover depends on the activities of all substrates and products, in contrast to the enthalpy efficiency. Therefore, there is no single value for any given pathway, even under the conditions of full coupling as assumed in the comparison of enthalpy and Gibbs energy efficiencies (Table 1). In several invertebrates under anoxia the Gibbs force of phosphorylation declines to 44 $kJ.mol^{-1}$ ATP, and it is around 50 to 52 $kJ.mol^{-1}$ in many aerobic tissues (ref. 1). In aerobic muscle tissue at rest, the Gibbs force of phosphorylation can increase up to 72 $kJ.mol^{-1}$ ATP, whence the ergodynamic efficiency would increase to 92% if mitochondria were fully coupled at rest (ref. 24). The limitations of this assumption due to uncoupling at rest is well recognized (ref. 25), yet a normalized phosphorylation/catabolic Gibbs force ratio (force efficiency) of 0.92 can only be attained at high degrees of coupling (ref. 13).

The Gibbs energy efficiency has a theoretical maximum value of 1.0, but maximum efficiency is diminished with decreasing degree of coupling (ref. 13). Further limitations of optimum efficiency for maximum power output have been discussed on the basis of

flux/Gibbs force relations, where the effective driving force is the net of the normalized exergonic plus endergonic forces (ref. 4,26). At maximum efficiency, the exergonic force is fully compensated by the endergonic force, such that at "ergodynamic equilibrium" (ref. 4) of a fully coupled process the fluxes are zero (eq. 8).

Under exergy limitation, high efficiency and low metabolic flux and power can increase the potential for survival and reproduction. Then high efficiency and low power is an economy strategy which may be of selective advantage, as is recognized in studies of invertebrates under environmental anoxia (ref. 1). However, near maximum efficiency the diminution of the net Gibbs force is inhibitory for high flux, whence efficiencies decline under strenuous aerobic exercise (from 0.9 to 0.64). Compared to the high economy but low power succinate and propionate-acetate pathways, the low efficiency of the high-power lactate pathway is indicative of a general tradeoff between high power and maximum efficiency (Tab. 1).

Non-linear flux/force relations: kinetics and ergodynamics

In kinetics, the flow of a chemical reaction is called the "rate of conversion" [mol.s^{-1}] (ref. 12). In general, however, a *rate*, r, should have the dimension *per unit of time* [s^{-1}], and the general relation between rate and flux is,

$$r = \frac{1}{Y}\frac{dY}{dt} \tag{26}$$

where Y is an amount per unit system size (an "activity" or "concentration") and $dY.dt^{-1}$ is the flow per unit system size, as defined in eq.(2). At constant Y, a change in flux is directly proportional to a change in rate. Therefore, a linear flux/force relation (see eq. 8),

$$\frac{dY}{dt} = -L\ F_Y \tag{27}$$

is also a linear rate/force relation, where b is the phenomenological rate coefficient,

$$r = -b\ F_Y \tag{28}$$

Substituting for r in eq.(28) from eq.(26) and solving for the flux yields (ref. 27),

$$\frac{dY}{dt} = -b\ Y\ F_Y \tag{29}$$

where the conductivity, L (eq. 27), is explicitely given as a function of the "activity" term Y, $L = b\ Y$. The form of eq.(29) suggests a possible reconciliation between kinetics and nonequilibrium thermodynamics, since it contains both an "activity (concentration)" term and the driving force (ref. 27). One of the unresolved problems in the analysis of chemical

flux/Gibbs force relations is the prediction of the dependence of the conductivity on total concentration (ref. 6), and the fact that linear flux/force relations are observed in bioenergetics far beyond the linear range predicted by kinetics (ref. 6,27).

The theory of the ergodynamic variables exerting control over coupled metabolic fluxes, complementary to specific enzyme kinetic mechanisms, is still incomplete. Ultimately, these metabolic energy conversions must be related to biological growth (ref. 28). The analysis of growth efficiency, however, requires the distinction between internal exergy (energy) transformation and external transfer, two components in the analysis of systems and processes.

SYSTEMS AND PROCESSES: EXERGY TRANSFER AND TRANSFORMATION

In chemical thermodynamics, experimentation with closed isothermal systems provides us with an elegant simplification, since then the parameter changes due to *processes* of exergy *transformation* in chemical reactions or phase transitions (dG etc.) are identical to the parameter changes of the *system* (dG_{Sys} etc.; Fig. 1). The bound energy of the transformation process (e.g. a chemical reaction) equals T times the entropy change of the system,

Closed isothermal system: $$T\ \mathrm{d}S_{Sys} = \mathrm{d}B \tag{30}$$

Negative entropy?

Living cells and organisms can in many cases be described as isothermal systems operating at constant pressure. But, of course, biological systems are open for the exchange of matter. Therefore, both energy transformation balances and external energy and mass transfer balances are required for the thermodynamic analysis of open systems (ref. 29). In ergodynamic terms, the entropy change of an open system is due to *internal* entropy production, $T\,\mathrm{d}_{int}S = -\mathrm{d}D$ (transformation), and *external* entropy exchanged with the environment, $T\,\mathrm{d}_{ext}S$ (transfer). This concept of an entropy balance (ref. 29),

$$T\ \mathrm{d}S_{Sys} = T\ \mathrm{d}_{int}S + T\ \mathrm{d}_{ext}S = -\mathrm{d}D + T\ \mathrm{d}_{ext}S \tag{31}$$

is implicit in Schrödinger's notion of "negative entropy" applied to living organisms (ref. 30), which require a *negative external entropy* for maintenance. At steady state, $\mathrm{d}_{int}S = -\mathrm{d}_{ext}S$. Either export of heat to the environment or import of chemical Gibbs energy in the form of food from the environment can be interpreted as negative external entropy (see also ref. 9). Without relying on the rather ambiguous terminology of entropy, the condition of reversibility ($\mathrm{d}D = 0$), the extent of irreversibility ($\mathrm{d}D < 0$) and the exergy balance of open systems,

dG_{Sys}, can be described on the basis of (internal) dissipated energy, dD, work, dW, and exergy exchanged in the form of matter, $d_{ext}G_m$ (Fig. 1 and 2). In general, the change of a system parameter (Sys) is the sum of internal (*int*) and external parameters (*ext*; ref. 29),

$$dG_{Sys} = d_{int}G + d_{ext}G \tag{32}$$

$$dH_{Sys} = d_{int}H + d_{ext}H \tag{33}$$

The first and second laws of thermodynamics are related to the *internal* parameters in eq.(33) and (32), by the observation that at constant pressure energy (enthalpy) is conserved,

$$d_{int}H = 0 \tag{34}$$

and that the dissipated energy is always lost to the system (always negative, or zero),

$$dD = d_{int}G \leq 0 \tag{35}$$

The choice of enthalpy and Gibbs energy instead of internal energy, U, and Helmholtz energy, A, implies that volume expansion $p\,dV$ is considered as an expansion of the system, and the pressure is kept constant. Without these restrictions, H and G should be substituted by U and A throughout this analysis.

Steady state and growth efficiency

The *external* parameters are of particular importance in open systems. They are the sum of the transfer of heat, work and matter (mass, m; Fig. 2),

$$dG_{Sys} = dD + dW + d_{ext}G_m \tag{36}$$

$$dH_{Sys} = dQ + dW + d_{ext}H_m \tag{37}$$

At steady state the changes of system parameters are zero, $dG_{Sys} = 0$ and $dH_{Sys} = 0$. Then the sum of all transformations at constant pressure is described completely by (Fig. 1),

$$dG \rightarrow dD + dW \tag{38}$$

Note the difference between the internal Gibbs energy, $d_{int}G$ (eq. 35) and the Gibbs energy of (internal) transformations, dG. The ergodynamic relation (eq. 38) describes the fate of the transformed exergy without defining the actual thermal changes. By comparison, the corresponding thermodynamic relation (eq. 39) is a formulation of the first law of thermodynamics on the conservation of energy (enthalpy) in terms of heat and work,

$$dH \rightarrow dQ + dW \tag{39}$$

Transformation and transfer can be described separately, although they are coupled to

various degrees, being fully matched at steady state. The Gibbs energy and enthalpy changes over dt due to transfer of matter are,

$$d_{ext}G_m = \sum_i d_{ext}n_i \mu_i \tag{40}$$

$$d_{ext}H_m = \sum_i d_{ext}n_i H_i \tag{41}$$

where $d_{ext}n_i$ is the amount of the ith substance transferred into the system in the time interval dt, and μ_i and H_i are the chemical potentials and partial molar enthalpies of the ith substance at constant temperature, pressure and at constant composition. The requirement of constant composition is met at steady state of the system. Then external transfer of any substance i is compensated by internal transformation in the reaction, $d_r n_i = -d_{ext}n_i$. A substance B added to the system, $d_{ext}n_B > 0$, becomes a substrate which is removed in the reaction, indicated by a negative stoichiometric number, $\nu_B < 0$. At steady state, the Gibbs energy and enthalpy of *transformation* (reaction; see eq. 20 and 21 for the molar quantities) are equal to the negative external Gibbs energy and enthalpy *transfer* (eq. 38 and 39),

$$-\sum_i d_{ext}n_i \mu_i = \sum_i d\xi \nu_i \mu_i = dD + dW \tag{42}$$

$$-\sum_i d_{ext}n_i H_i = \sum_i d\xi \nu_i H_i = dQ + dW \tag{43}$$

For a complete ergodynamic or thermodynamic input-output system analysis, the external parameters, dW, $d_{ext}G_m$ and $d_{ext}H_m$ are partitioned into their input and output components. Different efficiencies can be defined on the basis of these input and output components, depending on an emphasis on the changes of the whole system, on mass transfer, or on the regulation of exergy and energy transformations which govern the dynamics of the system.

During microbial or animal growth the work term can be taken as zero but, of course, during muscle performance work is the all important external output for calculating efficiency (ref. 23,31). When growth is described as a steady state process, then the invariant system (eq. 36 and 37) is the biomass, X [g, or mol organic carbon], which is considered as a constant catalytic unit. Converting the external fluxes of substrates and products to energy units (eq. 41), steady state heterotrophic growth per dt is described (from eq. 37),

$$0 = d_{ext}H_{in} + d_{ext}H_{out} + d_{ext}H_X + dQ \tag{44}$$

$d_{ext}H_X$ is the enthalpy of biomass produced per unit of biomass ([J.g^{-1}] or [J.mol^{-1} C_X] where C_X is organic carbon in biomass). At present, the Gibbs energy of biomass can at best be

approximated by the enthalpy with an unknown magnitude of error. This illustrates the importance of calorimetric measurements of heat flux in studies of growth, when assessing the dissipated energy, dD (the entropy production, eq. 31) by the heat (compare eq. 42 and 43). Such approximations are valid for aerobic but not for anoxic catabolism (ref. 1), and further research is required on exergy/energy relations in biomass. The following discussion will be restricted to aerobic growth.

Anabolic transformation and production of biomass

Contrasting expressions of efficiency are obtained, depending on the interpretation of input and output energy changes, $d_{ext}H_{in}$ and $d_{ext}H_{out}$ (eq. 44). One approach combines the external transfer (system) analysis (eq. 43, first expression) with transformation (process) analysis (eq. 43, second expression; see also Fig. 2). Then the external net input of catabolic substrates and products equals the enthalpy change of catabolic reactions (identical to dH_{r1} [J.g^{-1}] in eq. 23; catabolic half cycle, Fig. 3),

$$\frac{d_{ext}H_{in}}{dt} = {}_{k}J_{O_2}\,\Delta_{k}H_{O_2} \tag{45}$$

Internally the catabolic energy transformation, coupled to ATP production, is connected in series to the anabolic transformation, coupled to ATP consumption (Fig. 4). The output is the net of external substrate and biomass enthalpy, $d_{ext}H_{out} + d_{ext}H_X$, combining two steady state output parameters of eq.(44). The corresponding transformation includes the possible degradation (d) of complex substrates such as polymers and anabolic reactions (a, Fig. 4),

$$\frac{d_{ext}H_{out} + d_{ext}H_X}{dt} = {}_{ext}J_X\,\Delta_{da}H_X \tag{46}$$

${}_{ext}J_X$ is the net flux of biomass production, expressed in [g.s^{-1}.g^{-1}] or [mol C_X.s^{-1}.mol^{-1} C_X] which is the growth rate, μ [s^{-1}]. $\Delta_{da}H_X$ is the enthalpy change per unit of biomass produced in the transformation of external substrate to biomass (degradation and anabolism). Then the growth efficiency is the ratio of net anabolic transformation to catabolic transformation,

$$\eta_H = -\frac{{}_{ext}J_X}{{}_{k}J_{O_2}}\,\frac{\Delta_{da}H_X}{\Delta_{k}H_{O_2}} \tag{47}$$

This definition of growth efficiency is based on the partitioning of the substrate fluxes into catabolic and anabolic substrates (ref. 28). It can be compared to the efficiencies of ATP production in the catabolic compartment (eq. 23). The growth efficiency is the product of

the compartmental efficiencies (ref. 4,31). Depending on the degree of reduction of the external anabolic substrate relative to biomass, a positive or negative value of $\Delta_{da}H_X$ and of growth efficiency (eq. 47) is obtained. For several substrates the net efficiency is zero, e.g. when protein is consumed and deposited as protein. Then the input in eq.(47) equals the heat flux. On this basis the oxycaloric equivalent (see above) predicts heat flux accuarately during growth (ref. 19). However, the *internal* processes of anabolism, e.g. formation of peptide bonds, are endothermic and endergonic. Therefore, gross anabolic efficiencies are positive, comparable to the gross catabolic efficiency of ATP turnover. Only the internal ergodynamic efficiencies provide the necessary information on the control of anabolic flux and can be interpreted in terms of optimum efficiency (ref. 4). $\Delta_{da}H_X$ (or the corresponding Gibbs energy of reaction, ref. 28) reflects the nature of the external substrate which exerts a strong effect on growth efficiency (eq. 47), overriding or amplifying that of power or economy strategy. The importance of the degree of reduction of the growth substrate for growth efficiency is well known in aerobic fermentations (ref. 19).

A different apportionment of energy changes 'in' and 'out' (eq. 44) accounts for the input in terms of substrate flux times the partial molar enthalpy of the total substrate, H_S [J.mol^{-1} substrate relative to a reference level], rather than considering the enthalpy of the catabolic reaction [J.mol^{-1} extent of reaction] (eq. 21). Consequently, the output is the growth rate times the specific enthalpy of the biomass, H_X, different from the enthalpy of the anabolic (da) reaction per unit of biomass formed. Then growth efficiency is independent of the internal partitioning into catabolic and anabolic transformations. The external transfer efficiency is based on the external fluxes of biomass, ${}_{ext}J_X$, and organic substrate, ${}_{ext}J_S$,

$$ {}_{ext}\eta_H = \frac{{}_{ext}J_X}{{}_{ext}J_S}\,\frac{H_X}{H_S} \qquad (48) $$

Since enthalpies, H_X and H_S, are not absolute values, appropriate reference states must be chosen (ref. 32). This choice exerts a strong effect on the calculated growth efficiency (eq. 48). In contrast, eq.(47) is independent of the choice of reference states, since these cancel in the calculation of reaction enthalpies (eq. 21). Enthalpies of formation are used for H_i in the calculation of enthalpies of reactions (eq. 21), but enthalpies of combustion are conventionally considered (ref. 33) in the context of growth efficiency and energy budget calculations in biological energetics. Maximum microbial growth efficiencies (eq. 48) are 0.6 (ref. 19). The choice of enthalpies of combustion can be rationalized for aerobic growth since then the external enthalpies of transferred substrates, H_S, approximate closely the actual enthalpies of transformation in the catabolic reaction (eq. 43).

In animal energetics the external growth efficiency (eq. 48) is known as the gross production efficiency, P/C, the ratio of produced biomass energy, P, and consumed food energy, C (ref. 34). These components of the energy budget of an animal, as well as the energy loss in faeces, F, are based on bomb calorimetry, whereas the respiratory heat loss, R, is the heat flux in the living animal (ref. 35). Losses in nitrogen excretion, U, are often considered insignificant. Then another interpretation is given to $d_{ext}H_{out}$, when eq.(44) is written step by step in the notation of animal energetics (ref. 35),

$$0 = C + F + P + R \tag{49}$$

where $C + F$ is the net assimilated energy, A. The net production efficiency is,

$$_{ext}\eta_{H^{(net)}} = -\frac{P}{A} = \frac{P}{P + R} \tag{50}$$

The commonly used P/R ratio (ref. 34), however, is the output of eq.(48) divided by the input of eq.(47). Various expressions of efficiency can thus be placed in the context of a more rigorous thermodynamic analysis. This is a basis for comparison of growth efficiencies in different areas of biological energetics.

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