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Chapter 7

EFFICIENCY AND POWER STRATEGIES UNDER HYPOXIA. IS LOW EFFICIENCY AT HIGH GLYCOLYTIC ATP PRODUCTION A PARADOX?

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I. INTRODUCTION

A. HIGH EFFICIENCY IS ADAPTIVE

"Efficiency and speed characterize the time value of the modern age".[11] Processes should be efficient, fast, and powerful. The term *efficiency* is used in common and scientific language with different meanings, and correspondingly vague definitions may lead to confusion. In bioenergetics, efficiency is strictly defined as the output/input power ratio, that is the fraction of useful energy (exergy) converted into work. By increasing efficiency, the invested exergy can be reduced at constant gain. As such, efficiency is an eminent resource which remains poorly exploited in present technology and society. Today, high efficiency is valued as an achievement and goal. In physiology and ecology high efficiency is considered to be adaptive.[2] Why, then, are efficiencies of technologically designed and biologically evolved processes limited?

B. HIGH POWER-LOW EFFICIENCY: AN EVOLUTIONARY PARADOX?

Phosphorylation of adenosinediphosphate (ADP) to adenosinetriphosphate (ATP) ranks among the most important energy transformations in the biosphere. In aerobic and anaerobic heterotrophic systems, ATP production is energetically driven by exergonic catabolic reactions at the expense of reduced organic substrates. Glycolysis to lactate yields 3 mol ATP per mol glycosyl-unit,[3] which is merely 8.1% of the aerobic coupling stoichiometry of 37 mol ATP per unit glycogen. At an efficiency of Gibbs energy conversion of c. 65%, lactate production represents a low efficiency pathway.[4]

The Gibbs-energy efficiencies in the high power mode are nearly identical in respiratory and glycolytic ATP production in skeletal muscle,[5] despite the more than tenfold difference of the ATP/glycogen stoichiometries. The low efficiency in these two contrasting high power pathways of aerobic and anoxic ATP production indicate the functional importance of optimizing (in contrast to maximizing) efficiency.

When the succinate-propionate-acetate pathways were discovered in euryoxic invertebrates,[2,6] the interpretation in terms of biochemical adaptation was readily at hand: the stoichiometric ATP/glycogen ratios are up to 2.1-fold higher compared to the lactate pathway. Efficiencies increase to >80%.[4] Obviously, high ATP stoichiometry and efficiency are of selective advantage under anoxia when power is low and energy is limited. This familiar explanation of increased efficiency is important, but it addresses only half of the problem: why are efficiencies of ATP production different in various pathways? Is there a functional role for low efficiency? Or is low efficiency at high glycolytic ATP and lactate production a paradox?

The notion of "increased" efficiency reflects the historical fact that the lactate pathway was discovered first. Decades later, the more efficient succinate-acetate-propionate pathways were investigated — hence "higher" efficiencies. Consider a reversed time course in scientific discovery: if Meyerhof would have worked on anoxic mussels (*Mytilus*) instead of frog muscles, he might have discovered succinate production. Then the stoichiometry of 4.73 mol ATP per mol glycosyl-unit in the succinate pathway[4,7] would serve now as the anoxic reference stoichiometry, observed in many invertebrates under environmentally induced *passive* anoxia. Subsequently in our imaginary history of comparative biochemistry, lactate production would be discovered with a "lower" ATP stoichiometry, leading to the question: what is the advantage of the "decreased" anoxic ATP gain at high power output? If high efficiency is adaptive, is therefore low efficiency maladaptive?

The high efficiency pathways are widespread in passive invertebrates and may well represent a phylogenetically early character. Diminution of efficiency appears to be an

evolutionary paradox, considering the low efficiency of ATP production in the high power lactate pathway operating in specialized skeletal muscle under extreme work loads. Why are the biochemically efficient pathways with the highest ATP yield not exploited under active anoxia, when ATP demand is highest?

This apparent evolutionary paradox can be resolved on the basis of nonequilibrium thermodynamics (*ergodynamics*). Classical thermodynamics defines maximum efficiency but is insufficient for explaining optimization strategies of energy transformation. An introduction to the relevant physicochemical parameters will (1) clarify the controversial notion of efficiency in the context of metabolic energy conversion. The biochemical ATP-stoichiometry and flux ratios are an important component of efficiency of catabolic ATP production. In addition, efficiency depends on the chemical potentials and forces calculated as the molar Gibbs energy changes under typical cellular conditions. (2) This leads to a discussion of the control of flux from the perspective of efficiency and coupling. ATP stoichiometries per se are insufficient for solving the paradox of the low efficiency lactate pathway. (3) The discussion of optimum efficiencies of energy transformation will provide the framework for a quantitative solution of the apparent paradox on the evolution of inefficient pathways for meeting high output demands. Maximization of power and efficiency of ATP production are mutually exclusive. An outline of ergodynamic principles in metabolic energy conversion in the catabolic-anabolic energy chain yields a quantitative concept for assessing the economical nature of metabolic design and the balance of ATP demand and ATP supply. This opens new insights into the network of biochemical adaptation, particularly relevant for hypoxia and anoxia under which conditions the extremes of high and low metabolic power are observed.

II. METABOLIC EFFICIENCY AND POWER IN BIOENERGETICS

The relation between efficiency and physiological adaptation is addressed in many contexts. Even the "lactate paradox" described in high altitude physiology may be fundamentally connected to (aerobic) efficiency.[8] Energy transformation between coupled input and output processes requires efficiency. Ergodynamic efficiency is the fraction of exergy obtained in the output (driven) process relative to the exergy invested in the input (driving) process. Efficiency is a function of the relation between input and output *forces* and of the tightness of coupling between the input and output *fluxes*. Power (Gibbs energy change per unit of time) is the product of flux and force. Complementary to the fluxes, the ergodynamic forces are quantified as a basis for calculating the power and efficiency of metabolic energy transformations.

In bioenergetics the fluxes are the rates of chemical reactions, ion fluxes across membranes, electric current densities, or velocities of mechanical movement in actin-myocin interactions. The corresponding forces are the molar Gibbs energies of reaction determined at cellular conditions, the chemical and electric potential differences across membranes, and the mechanical forces of movement. For instance, consider catabolic oxygen flux or the rate of oxygen consumption as the input flux, and the flux of ATP production as the output flux. The output/input flux ratio is the experimental ATP/O_2 ratio which is an important component of metabolic efficiency. In the biochemical literature the flux ratio is frequently even considered to be *the* expression of efficiency. However, it must be recognized that bioenergetic efficiencies are not merely molar ratios but express Gibbs energy ratios or power ratios. The catabolic power input is the product of oxygen flux and input force. The power output of ATP production is the ATP flux times the output force. The corresponding input and

output forces are the molar Gibbs energy of catabolic oxygen consumption and the molar Gibbs energy of phosphorylation. The efficiency can be regulated by changes in the flux ratio (e.g., uncoupling) and by changes in the force ratio (e.g., changes of the phosphorylation potential). Catabolic efficiency is functionally important in terms of the output flux of ATP production and in terms of the output force for maintaining a high phosphorylation potential as required for biochemical and physiological purposes.

A. AN UPPER LIMIT OF EFFICIENCY AND TIME AS A RESOURCE

Darwin's theory of evolution establishes a historical perspective in the natural sciences by its focus on selection as a "driving force" of biological change in time. Similarly, an emphasis on time is introduced into "finite time thermodynamics" by analysis of the causal relation between ergodynamic forces and fluxes in irreversible processes.[9] The time-exergy framework interrelates a variety of theories on dynamic systems, such as evolutionary theory, economics, technological engineering, ergodynamics, information theory, cybernetics, and chaos theory. In an interdisciplinary field with rapid transitions from conventional to mundane terminology, it is particularly important to evaluate traditional concepts and definitions, to be able to link them with specific contemporary developments.

The second law of thermodynamics, which may be called the *dissipation or entropy law*, sets the absolute theoretical limit on the maximum efficiency at 1.0. The second law provides a rigorous definition of efficiency in terms of "high grade energy" (exergy, useful work free energy, or Gibbs energy). Emphasis is placed on the distinction between energy (first law of thermodynamics) and exergy introduced by the second law. This implies a consideration of entropy (bound energy)[10] associated with the energy. Exergy is exclusively that quality of energy which can theoretically be converted into mechanical work. Exergy can be dissipated and is then irreversibly lost, whereas energy is always conserved in the transformation between various forms and qualities of energy and in the flow of energy between a system and its surroundings. In chemical thermodynamics, the difference between enthalpy, ΔH, and Gibbs energy, ΔG, reflects the distinction between energy and exergy. In turn, thermodynamic or thermal efficiency, η, is based on energy, whereas *erg*odynamic or power efficiency, ϵ, is always related to ex*ergy* (erg = work). Ergodynamic efficiency is the fraction of exergy converted into work, with a maximum value of one when no exergy (Gibbs energy) is dissipated.[11]

Actual ergodynamic efficiencies in biological Gibbs energy transformation are commonly well below unity. Classical thermodynamics defines the maximum theoretical limit of efficiency, but cannot rationalize the extent by which a process falls short of maximum efficiency.[12] Concepts of nonequilibrium thermodynamics (ergodynamics) address this important question in an evolutionary context, by contrasting *exergy and time* as limiting resources. High efficiency saves exergy, at the cost of time. High power output (= work per unit of time) saves time, at the expense and dissipation of exergy, leading to low efficiency. As a practical example consider the *economy speed limit* when driving a car. Different environmental conditions and functional demands exert specific selective pressures with divergent emphasis on *efficient* utilization of exergy in economy strategy or *effective* utilization of time in power strategy.[5]

The exclusion of high power and maximum efficiency is at its limit related to the concept of reversibility of classical thermodynamics. As the efficiency approaches 100%, the coupled process becomes fully reversible. Simultaneously, the net flux approaches zero, since only at zero net flux (velocity) all frictional, dissipative effects are annihilated. 100% efficiency causes coupled processes to stop. This can be visualized on a balance in *equilibrium* ("*equal measures of weight*") when the opposing gravitational forces counterbalance each other: there is no falling or lifting of balanced weights.

Optimum efficiency depends basically on two aspects:

1. on the relation between efficiency and power output. Maximum values of both cannot be achieved simultaneously. The relation between power and efficiency is a complex function of system design (morphology, physiology and biochemistry).
2. on the relative fitness values of the resources, exergy and time. Increased power output saves time but costs exergy. The exergy and time values are set by the interactions with the environment (behavior, ecology). In commerce, exergy and time are embodied in a common currency: money. A common currency is the essence of optimization theory and tradeoff models in evolutionary physiology and ecology.[13] Fitness is the obvious evolutionary criterion of merit. But fitness, as a currency of evolution, is difficult to measure. It is the result of optimization of survival, fecundity, and generation time. Fitness depends fundamentally on the balanced investment of exergy and time, on optimized metabolic efficiency and power.[14]

Maximum metabolic power output[15] is an oversimplification as a general criterion of "thermodynamic optimality" which would apply only under conditions of sustained effluence. Owing to overexploitation of resources and diminution of carrying capacity, maximum power strategy (*r*-selection) converts into poor fitness measured as the propagation of this strategy through subsequent generations in a densely populated environment.[5] *K*-selection under resource limitation does not produce phenotypes with maximum metabolic power, else the biosphere would consist of ever faster biological racing machines.[14] An understanding of the physicochemical basis and the physiological trade-offs of metabolic efficiency is essential for a firm scientific establishment of the concept of metabolic adaptation.

B. FUNDAMENTAL COMPONENTS OF METABOLIC EFFICIENCY

1. Gibbs Energy and Power

The "definition of efficiency given by classical equilibrium thermodynamics is inadequate to furnish a result of practical interest for mitochondria" since it is "confined to the forces involved in the process of oxidative phosphorylation and neglects the flows".[16] The nonequilibrium thermodynamic formalism defining efficiency as a power ratio[17] is invoked for the imperative consideration of both forces and fluxes. The postulated limitation of classical thermodynamics to furnish an unequivocal definition of efficiency, however, is in remarkable contrast to the fundamental relation between efficiency and the second law (of classical thermodynamics). Consider the definition of efficiency, ϵ, as the output/input ratio of Gibbs energy (exergy) changes measured over a common time interval,

$$\epsilon = -\frac{d_{tr}G_{out}}{d_{tr}G_{in}} = -\frac{P_{out}}{P_{in}} \qquad (1)$$

Exergy transformation (tr), $d_{tr}G$, per unit of time, dt, is power (Equation 2). Therefore, exergy efficiency and power efficiency are identical. A conflict does not exist between the exergy efficiency of classical thermodynamics and power efficiency of nonequilibrium thermodynamics. Caution is required not to be misguided by inappropriate terminology.

Efficiency is a dimensionless quantity [$J \cdot J^{-1}$ or $W \cdot W^{-1}$], with a maximum of 1. In a completely uncoupled respiratory chain no ATP is produced (zero mitochondrial output flux) and the output Gibbs *energy* change, $d_{tr}G_{out}$, is zero. In this case, the efficiency of mitochondrial exergy conversion (Equation 1) is zero, independent of the output *force* measured by the phosphorylation potential.

Power [W = J · s^{-1}] is exergy [J] per unit of time [s],*

$$P = \frac{d_{tr}G}{dt} = J\,F \tag{2}$$

The definition of power commonly used in ergodynamics is the product of flux, J, and force, F (right-hand side of Equation 2). Importantly, metabolic power is not identical to metabolic flux. Catabolic oxygen flux is converted to catabolic power by multiplication by a force, the Gibbs energy change per mol O_2 consumed, approximately -480 kJ · mol^{-1} O_2. Correspondingly, mechanical power is not identical to speed. The forces are equally important. Metabolic power can be measured by direct calorimetry as heat dissipation per unit of time (heat flux) when catabolism is fully aerobic, but heat flux underestimates the catabolic power of anoxic pathways.[4] Again, the corresponding force must be considered to convert thermodynamic heat flux into ergodynamic power.[10] Muscle physiology apart, most studies on physiological and biochemical adaptations are confined to the fluxes.

2. Gibbs Energy and Force

Confinement to the forces and neglect of the fluxes is implied in a definition of efficiency if chemical forces rather than Gibbs energies are used in Equation 1. Then the critique of the definition of efficiency[16] appears to be correct. However, this neglects the distinction between Gibbs energy change, $d_{tr}G$, and force, F (Equation 2). The contrast is obscured in most textbooks by reference to the chemical *force* as molar Gibbs *energy*. The *molar* reaction Gibbs energy is the driving force of a reaction. Importantly, Equation 1 is *not* based on *molar* Gibbs energies. The Gibbs energy efficiency derived from classical thermodynamics does implicitly account for the fluxes. This view warrants careful explanation.

Force and Gibbs energy are contrasted as intensive and extensive properties, respectively (see footnote in Section II.B.1). The fundamental distinction between exergy and force is underscored at the level of *SI* units for mechanical force [J · m^{-1} = N] and electric force or voltage [J · C^{-1} = V]. Corresponding to the lack of clarity in the distinction between Gibbs energy and force, a separate unit for chemical force[10] is unfortunately not yet incorporated into the system of *SI* units.[18] Electric energy (exergy [J]) is force [J · C^{-1}] times the amount of electrons or charge [C] (electrical advancement). Gibbs energy (exergy [J]) is chemical force [J · mol^{-1}] times the amount of reacting substance [mol] (reaction advancement). Let the advancement of (electro)chemical reactions be $d_r\xi$ (mol per unit system size), such that any flux, J, is[11]

$$J = \frac{d_r\xi}{dt} \tag{3}$$

Then exergy transformation is the product of advancement, $d_r\xi$, and the corresponding force, F (Equation 4). The power of transformation, P_r, is flux times force (compare Equations 2 and 5),

$$d_rG = d_r\xi\,F \tag{4}$$

* *Extensive quantities*, such as power, P [W], Gibbs energy of reaction, d_rG [J] or the amount of substance B in a reaction, d_rn_B [mol], may be expressed *per unit of system size* (volume or biomass) throughout the text, without introducing a different name or symbol. Strictly, power per volume should be indicated as P_V. The size of biological systems is frequently quantified in terms of dry biomass. Then the units of the size-specific quantities P, d_rG, and d_rn_B are [W · g^{-1}], [J · g^{-1}], and [mol · g^{-1}].

$$P_r = \frac{d_r\xi}{dt} F \tag{5}$$

In catabolic energy transformation, the Gibbs energy output in the phosphorylation reaction, d_pG (Equation 1; see footnote in Section II.B.1), is calculated by multiplication of (1) the *amount* of ATP produced in substrate level phosphorylation and electron transport, $d_r\xi = d_pn_{ATP}$, and (2) the output *Gibbs force* of phosphorylation, $F = \Delta_pG_{ATP}$ [kJ · mol^{-1} ATP] (Equation 4),

$$d_pG = d_pn_{ATP}\,\Delta_pG_{ATP} \tag{6}$$

To avoid confusion between Gibbs energy change, d_pG, and force, Δ_pG_{ATP}, the latter should be termed *Gibbs force*.[10,11] This is more appropriate than the conventional term *molar reaction Gibbs energy*. The output force is the exergy per mol ATP produced. The Gibbs force is the partial derivative of Gibbs energy ($F = \partial G/\partial_r\xi$; Equation 4), at constant temperature and pressure,

$$\Delta_pG_{ATP} = \left(\frac{\partial G}{\partial_p n_{ATP}}\right)_{T,p} \tag{7}$$

In practice, the Gibbs force for cellular conditions is calculated from the standard Gibbs force (equilibrium constant) and the phosphorylation potential (mass action ratio),

$$\Delta_pG_{ATP} = \Delta_pG'^{o}_{ATP} + RT \ln\left(\frac{[ATP]\,c^o}{[ADP][P_i]}\right) \tag{8}$$

[ATP], [ADP], and [P_i] are the sum concentrations of adenosine triphosphate, adenosine diphosphate, and inorganic phosphate (including protonized and Mg-complexed species). c^o is the standard concentration (1 mol · dm^{-3}), $\Delta_pG'^{o}_{ATP}$, is the standard Gibbs force at specified pH, magnesium activity, temperature, and unit activity of ATP, ADP, and P_i (Figure 1). The Gibbs force of phosphorylation is routinely used in ^{31}P-NMR studies for calculating free ADP concentrations from Equation 8, assuming equilibrium between the creatine kinase reaction and phosphorylation of ADP.

Depending on the equilibrium constants reported in the literature since 1969, the calculated standard Gibbs force varies by more than ±2 kJ · mol^{-1}. $\Delta_pG'^{o}_{ATP}$ increases with pH above pH 6.5 and increases with decreasing Mg^{2+} activity in the range of 10 to 0.001 mmol · dm^{-3} (pMg of 2 to 6; Figure 1). The temperature dependence is comparatively small, at an increase of 1 kJ · mol^{-1} when the temperature is changed from 15 to 37°C. At 2 mmol · dm^{-3} Mg^{2+} activity (pMg = 2.7), $\Delta_pG'^{o}_{ATP}$, changes only slightly between pH 6.5 and 7.0, from 32.8 to 33.6 kJ · mol^{-1} ATP (Figure 1; square and circle, referring to cellular states I and II, see below).

Changes of the cellular ATP/ADP ratio and inorganic phosphate exert a strong effect on the actual output force. The concentration dependent term (the additive term in Equation 8 on the right) increases from 21 to 28.5 kJ · mol^{-1} with an ATP/ADP ratio increasing from 5 to 100 at 1 mmol · dm^{-3} P_i. This yields Gibbs forces of phosphorylation in the range of 55 (state I) to 62 kJ · mol^{-1} at pH 7. Under anoxia, ATP/ADP ratios and pH values tend to drop simultaneously, and anoxic Gibbs forces of phosphorylation decline (state II: 48 kJ · mol^{-1} at an ATP/ADP ratio of 1.5, 3 mmol · dm^{-1} P_i and pH 6.5).

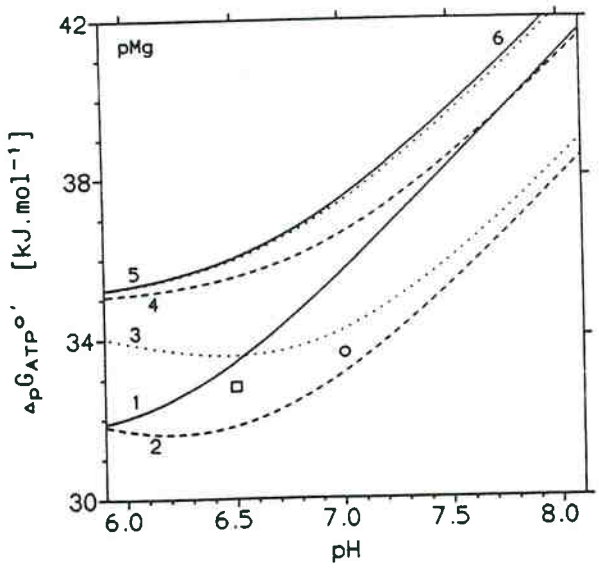

FIGURE 1. Standard Gibbs force of phosphorylation of ADP to ATP, $\Delta_pG'^{o}_{ATP}$ [kJ · mol^{-1}], at sum concentration of ATP, ADP, and P_i of 1 mol · dm^{-3}, as a function of pH and pMg (pMg = $-\log[Mg^{2+}]$). pMg values (numbers) correspond to free Mg^{2+} activities of 0.1 mmol · dm^{-3} (pMg = 1) to 1 μmol · dm^{-3} (pMg = 6). For the reaction

$$ADP^{3-} + HPO_4^{2-} + H^+ = ATP^{4-} + H_2O$$

the standard Gibbs force at 25°C (RT = 2.479 kJ · mol^{-1}) is[41]

$$\Delta_pG^o_{ATP} = -RT \ln K = -3.4 \text{ kJ} \cdot \text{mol}^{-1}$$

The equilibrium constants for the binding of H^+, Mg^{2+}, and Ca^{2+} are from References 41 and 42. Calculations are for pCa = 6 and ionic strength of 1 to 2 mmol · dm^{-3}. The circle and square are the values at pMg = 2.7 corresponding to states I and II (see text).

3. Force, Advancement of Reaction, and Flux

The input flux of aerobic metabolism is routinely measured as catabolic oxygen flux. The corresponding Gibbs force, $\Delta_kG_{O_2}$, is the partial derivative of Gibbs energy per unit oxygen consumed in the catabolic reaction k. Consider catabolism of glycogen (k) and phosphorylation of ADP (p) as the input and output reactions,

$$\text{p, } \nu_{ATP} = 1: \qquad 0 = -\text{ADP} - P_i + \text{ATP} + H_2O \tag{9.p}$$

$$\text{k, } \nu_{O_2} = -1: \qquad 0 = -1/6 \text{ Glyc} - O_2 + CO_2 + 5/6\ H_2O \tag{9.k}$$

The Gibbs force per glycosyl unit, Δ_kG_{Glyc}, is -2800 to -2900 kJ · mol^{-1} Glyc in aerobic respiration. Division by 6 yields $\Delta_kG_{O_2}$ (Equation 9.k) of -470 to -480 kJ · mol^{-1} O_2. For quantification of fluxes and forces not only the *type* of reaction but the stoichiometric *form* must be defined in terms of stoichiometric numbers, ν_i. These are the numbers preceding the symbols for each substance in Equation 9, 1 and -1 in most cases. ν_i is positive for products and negative for substrates.

A general definition of Gibbs force requires reference to the stoichiometry and particularly to the advancement of reaction, $d_r\xi$.[18] The Gibbs force of a defined reaction r, Δ_rG_B [kJ · mol^{-1} B], is the partial derivative of Gibbs energy per advancement, at constant temperature and pressure (compare Equation 4),

$$\Delta_rG_B = \frac{\partial G}{\partial_r\xi_B} = \nu_i \frac{\partial G}{\partial_r n_i}; \qquad |\nu_B| = 1 \tag{10}$$