The strong negative correlation between glycolytic flux and intracellular ATP concentration observed in yeast has long been an intriguing and counterintuitive phenomenon, which has been referred to as the ATP paradox. Herein, using principles of irreversible thermodynamics it was shown that if the ATP-consuming pathways are more sensitive to extracellular glucose than glycolysis, then upon glucose addition glycolysis performance can switch from an efficient working regime to a dissipative regime, and vice versa, depending on glucose availability. The efficient regime represents a good compromise between high output power and low dissipation, whereas the dissipative working regime offers a higher output power although at a high glucose cost. The physiological and evolutionary implications of this switch strategy are discussed.

Glycolysis is a metabolic pathway providing cells with energy in the form of ATP, which can be used to drive cellular processes. Thus, glucose is a metabolic substrate acting as an extracellular source of free energy powering life. In yeast, a strong negative correlation between glycolytic flux and intracellular ATP concentration has been widely documented (1–3). Because glucose catabolism is the main source of ATP-derived free energy, this observation (the higher the rate of glycolysis the lower the ATP content) is a counterintuitive phenomenon, which has been referred to as the ATP paradox. A sudden transition from glucose limitation to glucose excess leads to a new steady state at increased glycolytic flux with a sustained decrease in the ATP levels (3). Because ADP phosphorylation is tightly coupled to glucose breakdown, a higher glycolytic flux implies that more ATP is being formed. The subsequent increase in the flux throughout ATP-consuming reactions was thought to be brought about by an increased ATP/ADP ratio. Therefore, the decreased steady state level of ATP constitutes an intriguing observation. Somsen et al.(3), in an elegant work employing metabolic control analysis and a simple model, concluded that the experimental ATP changes can only be explained if the sensitivities of ATP-consuming routes to extracellular glucose are higher than that of the glycolytic pathway. Although this particular sensitivity of anabolic pathways solves the problem of how a drop in ATP concentration triggered by high glucose levels is possible, one question still remains open. What are the physiological implications of this behavior we have termed as the ATP paradox? In other words, what is the purpose of the stronger sensitivity to the level of extracellular fuel exhibited by ATP-utilizing pathways with respect to that of ATP-producing pathways?

Herein, using principles of irreversible thermodynamics it is shown that the glycolytic pathway can perform under two different thermodynamic regimes. One is optimal for obtaining a maximum output power from glucose degradation, although this high output power is at the expense of a high degree of dissipation. Thus, this regime is referred to as dissipative maximum output power (DMOP). The other regime represents an excellent compromise between high output power and low dissipation, so it is named efficient maximum output power (EMOP). More interestingly, it will be shown that the ATP paradox can be the expression of a molecular mechanism linking, throughout the intracellular level of ATP, the signal (low or high fuel levels) with the appropriate thermodynamic regime (EMOP or DMOP, respectively).

**THEORY**

_Evolutionary Trade-off between Efficiency and Rate_—In the energetic metabolism, for fundamental reasons that will be introduced later, there is always a trade-off between efficiency and rate (4, 5). Thus, thermodynamic analyses have concluded that a dissipative use of substrates may help cells to grow fast (6, 7). In fact, rapidly dividing cells have been described by some authors as dissipative devices (8). Although the purpose of this apparent waste of fuel has been a matter of discussion for a long time (9), it seems clear now that this dissipative use of fuels can be understood as the cost the organism must pay to grow rapidly (6). It seems understandable that under conditions of abundance, evolutionary pressure may have resulted in the selection of the fastest growing cells (dissipative genotype).

On the other hand, for most of other cells living in environments where energetic limitation is an important factor introducing metabolic constraints, the evolutionary pressure might be different. A cell exhibiting an efficient metabolism at the expense of a low rate, can produce more offspring from a given amount of resources. Hence, a selection favoring those cells able to grow as fast as possible but at optimal efficiency (efficient genotype) would be expected during periods of famine. One could argue that in an ecosystem containing a heterogeneous population of cells in resource competition, the fastest growing cells always will outgrow the competitors that grow more efficiently. Although only those cells that consume the resource more rapidly benefit from the higher rate of ATP production, all competitors exploiting the resource share the consequences of the more rapid fuel exhaustion. However,
Pfeiffer and Bonhoeffer (10) have drawn an evolutionary scenario where the cooperative use of external energy resources may have evolved in spatially structured environments favoring efficient genotypes. We then expect that the properties of ATP-producing and -consuming pathways have been under strong selection pressure during evolution. Hitherto, most of the studies have focused their attention on those conditions favoring one or other genotype and on analyzing the advantages and disadvantages of each one (4, 7, 10–12). Nevertheless, an interesting and unexplored possibility is to consider an organism endowed with a genotype enabling the cell to commute between dissipative and efficient glycolytic phenotypes depending on the environmental conditions. This strategy would be especially suitable for cells that live, or have evolved, under highly variable conditions as the case for most unicellular organisms of free life exposed to environments that have rapidly changing substrate availability. The remainder of this article will be devoted to investigate this possibility.

A Linear Irreversible Thermodynamic Approach to Glycolysis—Because we are only concerned with a phenomenological description of the energetics of glycolysis, we do not need to make any assumption on the reaction network. In other words, herein, glycolysis must be understood as a free energy transducing process (see Fig. 1). Thus, the only thing we have to know is that the splitting of glucose is an exergonic process strictly coupled to the phosphorylation of ADP to form ATP, which is an endergonic process. In the framework of irreversible thermodynamics, the rate of ATP production (J_1) is related to its conjugated force (X_1). Similarly, the flow from glucose to ethanol or lactate (J_2) is driven by the corresponding force (X_2). The generalized forces X_1 and X_2 can also be referred to as chemical affinities (equal to minus the actual change in free energy) and can be interpreted as the distance from equilibrium of each process. As known from irreversible thermodynamics, close to equilibrium one can be confident that the flows J_i are linear functions of the forces X_i. To accommodate multiple fluxes and forces, the equation may be generalized as

\[ J_i = \sum_{j=1}^{n} L_{ij} X_j \]  

(Eq. 1)

although far from equilibrium we lose the mathematical guarantéé of linearity (13), it does not mean at all that linear flow-force relations cannot be established. In fact, from an empirical analysis of different cellular processes, a linear relation is often found between the flow and the driving force, even if the process operates far from equilibrium (14–16). To explain these findings some authors have suggested that because linear flow-force relations would be identical to the near equilibrium flow-force relations (17), van der Meer et al. (18) showed that this might be a consequence of the particular kinetics of enzyme-catalyzed reactions. Westerhoff and van Dam (13) also analyzed diverse kinetic expressions, and in all cases they found that for a certain range of forces a linear relation could be assumed, suggesting that nearly linear relations between fluxes and forces are much more trivial than perhaps expected. In any event, the assumption of linear relations in the case of glycolysis is not without precedents (4, 5, 12, 19, 20). Focusing on the essential, our core model also considers a load imposed on glycolysis by the ATP-utilizing reactions (Fig. 1). As pointed by Stucki (14), it is reasonable to assume that the ATP-utilizing process is driven by the phosphate potential (−X_3). Thus, we have three fluxes that are linearly dependent on two forces, J_1 = L_11X_1 + L_12X_2 (Equation 2), J_2 = L_21X_1 + L_22X_2 (Equation 3), and J_3 = −L_31X_1 (Equation 4).

To have a steady state, we shall consider X_2 a constant parameter. Although steady states can also be obtained by setting J_2 or J_3X_2 constant, the X_2 constant seems to be the case in many biological systems (12, 21). A variation of the glucose level in the medium, for instance, would mean a change for the parameter X_2, which will lead to a new steady state. The validity of Onsager’s reciprocity theorem is also assumed, which establishes the symmetry of the matrix of phenomenological coefficients. Hence, L_21 = L_12. For this sort of linear converter, Kedem and Caplan (22) have introduced some useful concepts such as the degree of coupling, q = L_12L_11L_22 (Equation 5), which represents a dimensionless measure of how tightly the driven process is coupled to the driver process. The absolute value of the coupling coefficient will always be between 0 and 1. For glycolysis, where glucose degradation and ADP phosphorylation are tightly coupled, we can take q = 1. Another interesting concept is the so-called phenomenological stoichiometry Z = \frac{L_{11}}{L_{12}/L_{22}} (Equation 6).

A physically meaningful definition of the thermodynamic efficiency is, as proposed by Kedem and Caplan (22), the ratio between the output power of the driven process and the input power of the driver process \eta = −J_1X_1/J_2X_2 (Equation 7). It may also be convenient to normalize the output force (X_2) with respect to the input force (X_2) by defining the force ratio as x = ZX_1/X_2 (Equation 8). Hence, the force ratio can be seen as a relative, dimensionless, measure of the output force. Now, we are in conditions to express J_1, J_2, J_3, and \eta as explicit functions of the normalized variable x (see supplemental material), J_1 = ZL_{12}X_2(x + 1) (Equation 9), J_2 = L_{22}X_2(x + 1) (Equation 10), J_3 = −(L_{32}X_2/Z)x (Equation 11), and \eta = −x (Equation 12). Within the driving region, where the endergonic process 1 (substrate-level phosphorylation) is driven by the exergonic process 2 (glucose splitting), the domain of all these functions lies between the origin and −1, x taking the value −1 only when the system is at equilibrium, and the change in free energy for the global process is zero (ZX_1 + X_2 = 0). Once we have established a core model for glycolysis, which has been described using a linear irreversible thermodynamic approach, we are in a position to further explore the working regimes that are accessible to our system.

Glycolytic Working Regimes—Above we have advanced the concept that energetic metabolism can perform either under a dissipative and fast regime (DMOP) or under an efficient although slower regime (EMOP). With the description of glycolysis as a free energy converter connected to a cellular load, it should be possible to identify and analyze the specific functions characterizing these thermodynamic regimes. Furthermore, because we have also noted that the phosphate potential can play a major role in determining the optimal working regime, the force ratio will be a convenient variable for these thermo-
dynamic functions. The remainder of this section will be devoted to analyzing the restrictions imposed on \( x \) to satisfy each of the considered working regimes. Then, the comparison of these theoretical values with the in vivo observed force ratio, under conditions either of resource abundance or scarcity, will help us to draw conclusions regarding the ATP paradox.

As deduced by simple inspection of Equation 9, the flow of ATP synthesis is maximal when the phosphate potential vanishes, \( x = 0 \). Hence this maximal rate of ATP production is of doubtful biological interest. Alternatively, one could hypothesize that instead of the flow of ATP formation, it is more convenient to maximize the phosphate potential \(-X_1\). However, from Equation 9 it becomes obvious that this potential is highest \((x = -1)\) when the rate of ATP synthesis is reduced to nil \( (f_1 = 0) \). This again lacks any physiological sense. The conclusion to be drawn from this trivial analysis is that despite the fact that rapid ATP formation is an important task for glycolysis, the pathway may have evolved to optimize working regimes other than maximum flow or maximum phosphate potential.

When considered alone, neither \( f_1 \) nor \( X_1 \) are variables that must take extreme values. In contrast, their product defines a new function referred to as output power \( P_o \), whose optimization by natural selection may be of biological interest as seen here, \( P_o = -J_1 X_1 = -L_{22} X_2^2 (x^2 + x) \) (Equation 13). Now, the value of \( x \) can be calculated, \( dP_o/dx = -2 L_{22} X_2^2 (2x + 1) = 0 \) (Equation 14), for which a maximum output power regime is achieved. Thus, the output power exhibits an extremum for \( x = -1/2 = x_{DMOP} \) (Equation 15), because in \( d^2P_o/dx^2 = -2 L_{22} X_2^2 < 0 \) (Equation 16) this extremum is a maximum.

In the foregoing, we have maximized \( P_o \) without considering the energy costs to obtain a maximal output power; for that reason this state is described as dissipative (DMOP). However, as pointed out by Maddox (23), for biological systems a high degree of fitness implies not only high output powers but also high efficiencies, low entropy productions, and low energy consumption rates, requirements that are considered next.

We looked for a function representing a compromise between high output power and low fuel cost. In this sense, Stucki (14) has proposed that the appropriate parameter to introduce the constraints of minimal energy costs into the previous optimization problem is the efficiency, which can be considered as a weight on the function \( P_o \). \( \eta = L_{22} X_2^2 (x^2 + x) \) (Equation 17). The value of force ratio permitting an EMOP can be found by the maximization of the above function, which occurs at \( x = -2/3 = x_{EMOP} \) (Equation 18).

**Conductance Matching**—Although the term “conductance matching” was originally coined by Stucki (14) to describe the condition enabling oxidative phosphorylation to operate in steady state at optimal efficiency, in what follows we would like to extend the application of this term to indicate the necessary and sufficient conditions to be satisfied to have glycolysis performing in the steady state and optimal \( \Omega \). Omega being either the dissipative output power function (Equation 13) or the efficient output power function (Equation 17).

Because our immediate aim is to analyze DMOP- and EMOP-working regimes operating under stationary conditions, we shall determine the constraints imposed on \( x \) to allow the system to operate under steady state conditions. For this purpose, Prigogine’s theorem of minimum entropy production (MEP) can be useful. This celebrated theorem claims that in a steady state, the entropy production rate shows a minimum value. The dissipation function, defined as the rate of entropy production, can be formulated as the sum of products of all fluxes and their corresponding driving forces (24). In our model the dissipation function takes the form \( \psi = T dS/dt = J_1 X_1 + J_2 X_2 = J_2 X_2 \) (Equation 19). Substitution of Equations 2, 3, and 4 into Equation 19 yields \( \phi = T dS/dt = (L_{11} + L_{12}) X_2^2 = 2 L_{12} X_2 X_3 + L_{22} X_2^2 \) (Equation 20). Bearing in mind the definitions of coupling degree (Equation 5), phenomenological stoichiometry (Equation 6), and force ratio (Equation 8), a straightforward calculation (see supplemental material) leads to \( \phi = L_{22} X_2^2 [1 + L_{22}/L_{11}] x^2 + 2x + 1 \) (Equation 21). Because under steady state conditions \( \phi \) displays a minimum, the force ratio...
must satisfy the equation given by $d\sigma/dx = 0$, $x = -1/(1 + L_{33}/L_{11}) = x_{\text{MOP}}$ (Equation 22).

If the system is to operate under a DMOP regime installed in a steady state (minimum entropy production), there is a double condition derived from equating Equations 15 and 22, that needs to be satisfied, $-1/(1 + L_{33}/L_{11}) = -1/2$ (Equation 23).

To obtain now the conductance matching allowing the system to perform at EMOP in steady state, we have to resolve the system of equations formed by Equations 18 and 22, $-1/(1 + L_{33}/L_{11}) = -2/3$ (Equation 25) and $L_{33} = \frac{3}{2}L_{11}$ (Equation 26).

The double conditions, in terms of force ratio and conductance matching, which the system must satisfy to operate in steady state under either DMOP or EMOP regime, are summarized in Table I. These conditions must be born in mind when addressing the corresponding conductance. If, as proposed by Somsen et al. (3), upon glucose addition the anabolic pathways are stimulated in ways other than via ATP level and more strongly than glycolysis, then a logic consequence might be a suitable change in the conductance matching allowing the transition from EMOP to DMOP.

Furthermore, this transition from EMOP to DMOP after glucose addition also predicts the decrease in the ATP chemical potential (see force ratios in Table I) that is observed in yeast and that we have referred to as the ATP paradox. One important difference between mammalian cells and yeast cells is the relative constant glucose concentration encountered by the former as compared with that encountered by the latter. Hence, we would like to suggest that reversible regulation of the working regime under which glycolysis performs might have evolved specifically to cope with sudden and large variations in the extracellular glucose concentration. If that is the case, a sustained decrease in the intracellular ATP concentration when extra glucose is available may be a counterintuitive phenomenon, but it makes indeed a great deal of metabolic sense.

Acknowledgment—We thank Miguel Angel Medina for his comments on the manuscript.