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OSCILLATIONS AND EFFICIENCY IN GLYCOLYSIS *

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We suggest that temporal oscillations of concentrations of intermediates in biochemical reaction systems may enhance the efficiency of free energy conversion (reduce dissipation) in those reactions. Experiments on glycolysis are used to estimate the Gibbs free energy changes along the glycolysis mechanism, and to postulate a construct for the glycolysis "machine" which involves: the PFK reaction as the primary oscillophor; the GAPDH reaction as a phase-shifting device; and the PK reaction with the property of intrinsic oscillatory response at resonance with the driving frequency. Analysis of a simple reaction mechanism with these postulated properties shows that the conversion of free energy from reactants to products is more efficient in an oscillatory than a steady state operation. The efficiency of free energy conversion in glycolysis from glucose + ADP to products + ATP is estimated to be increased by 5–10% due to oscillations. This may have been relevant for the evolutionary development of oscillations such as in glycolysis, especially in anaerobic cells.

Abbreviations

ADP	adenosine diphosphate
ATP	adenosine triphosphate
NAD^+, NADH	oxidized and reduced nicotinamide adenine dinucleotide
F6P	fructose 6-phosphate
FDP	fructose 1,6-diphosphate
DAP	dihydroxyacetone phosphate
GAP	glyceraldehyde 3-phosphate
DPG	1,3-diphosphoglycerate
3PG	3-phosphoglycerate
2PG	2-phosphoglycerate
PEP	phosphoenolpyruvate
PYR	pyruvate
PFK	phosphofructokinase
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
PGK	phosphoglycerate kinase
PK	pyruvate kinase
P_i	orthophosphate

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1. Introduction

Glycolysis is the conversion of sugars into pyruvate which is then further degraded to alcohol (yeast fermentation), lactic acid (in muscles), or acetyl coenzyme A (in aerobic organisms). For each molecule of sugar there is a net production of two molecules of adenosine triphosphate (ATP). Under anaerobic conditions this is a cell's major energy supply, the conversion of ATP into ADP being the primary source of free energy in biological systems [1]. The overall efficiency of this ATP production is about 30% if we assume a value of 7.3 kcal/mol for ATP and relate this to the total free energy made available by sugar degradation, which is 52 kcal/mol in alcohol fermentation, or 47 kcal/mol in lactate formation during muscular activity [2].

There are several ways to enter the glycolytic pathway, starting from any of a number of sugars, or from

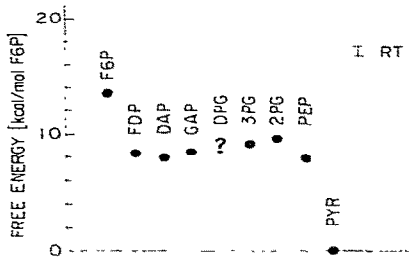

Fig. 1. Free energies of the various levels in glycolysis, relative to the value at the level of PYR. The data are for erythrocytes [9]. Note that for one molecule of F6P there are two molecules of GAP through PYR; thus the big drop between PEP and PYR refers to two moles of these species. The thermal energy RT is given for comparison. Values for DPG are missing because concentrations below 10^{-6} M could not be measured.

glycogen [2]. They converge at the level of fructose 6-phosphate which therefore marks the beginning of the universal part of the reaction sequence. Including the terminal product pyruvate there are altogether nine intermediates, see fig. 1. In all of the following we shall be concerned only with this central portion of the reaction mechanism which we view as a "machine" that transfers energy from one species (sugar) to another (ATP). A notable feature of its operation is that there are oscillations in the concentrations of chemical intermediates with periods of the order of a minute. These oscillations have been studied both experimentally and theoretically, and much has been learned thereby about the details of the enzymatic reaction mechanisms. In simplest terms, the observed oscillations are related to the feedback loops in the overall glycolysis reaction mechanism, for example product enhancement of enzymatic activity.

There arises now the question regarding the purpose, if any, of oscillations of concentrations in biochemical reactions. Feedback loops in a reaction mechanism, as in an electric circuit, assert some kind of control. In chemical systems there may be the need to control the level of certain concentrations or the rates of certain reactions, or the temperature (reactions couple with temperature through enthalpy changes as the reactions occur). It may therefore be argued that the need for such controls in biochemical processes is well documented, that the control is established by the introduction of feedback loops, and these loops, in turn, yield, as a by-product, oscillations of chemical concentrations. However, if control were the only purpose, then it might seem that the amplitude of the oscillations should be minimized as the quality of control is increased.

Alternatively, one may ask for a possible biological significance of metabolic oscillations. It is conceivable that they represent a special adaptation [3], and several such proposals have been made focusing on the adenylate energy charge. Goldbeter [4] argues that oscillations in the ATP/ADP/AMP ratio would alternately turn on the cell's energy producing and energy-consuming pathways, and thereby have them both more fully operative than if that ratio was held at an intermediate level. Tornheim in his discussion of glycolysis in muscle cells [5] suggests that the cell attempts to maintain a high ATP/ADP ratio under load condition, and that this can be more readily achieved in an oscillatory mode rather than in a steady state.

In this article we investigate the issue of the efficiency of reaction mechanisms, that is, we focus on the dissipative losses in the conversion of Gibbs free energy made available from the degradation of sugar to the formation of ATP from ADP. In doing so, we shall bear in mind that the systems of interest are driven far from equilibrium which implies that certain formalisms are not applicable which have been devised for close-to-equilibrium situations [6,7]. Since our investigation concentrates on oscillatory kinetics, it goes beyond Hills' analysis of free energy transduction in steady state situations [8]. In particular, we ask about dissipation in relation to chemical oscillations (which only occur far from equilibrium). We show that dissipation in glycolysis may be reduced, or the efficiency [‡] correspondingly enhanced, if oscillatory, as compared to the dissipation in a non-oscillatory stationary rate process, under the same average conditions. Hence there arises the possibility that chemical oscillations in biological systems represent an evolutionary advantage in that they allow for a less wasteful usage of the food (sugar) supply.

[‡] Dissipation is the *difference* between chemical power input and output, efficiency is the inverse *ratio*. This agrees with the analyses presented in refs. [6–8]. However, with more than one reactant and product involved, there is a freedom of choice in the identification of input and output sides, and in that respect our point of view differs from that developed in [6,7].

The net reaction of the central part in the glycolytic chain is [1] F6P + $2P_i$ + 3ADP + $2NAD^+$ → 2PYR + 3ATP + 2NADH + H^+ with a standard free energy change $\Delta G'_0$ = −13.9 kcal/mol. The actual free energy change as computed from the concentrations in a steady state situation is ΔG = −13.6 kcal/mol in human erythrocytes [9], or ΔG = −15.8 kcal/mol in yeast extracts [10]. This amounts to an average decrease of slightly less than 2 kcal/mol in each of the eight steps.

The actual free energy decreases do not, however, proceed in steps of equal height. Rather they occur mainly in the first and the last step of the sequence, with nearly equilibrium existing among the seven intermediates fructose diphosphate (FDP) through phosphoenolpyruvate (PEP). As this will be of central importance for our reasoning let us review the twofold experimental evidence: direct concentration measurements and phase relationships between the oscillating intermediates.

(i) Concentration measurements have been reported for ascites tumor cells [11], human erythrocytes [9], and yeast cell extracts [10]. Using the known standard free energies for the individual glycolytic reactions, we can convert these values into free energy profiles as shown in fig. 1 for the example of human erythrocytes [9,12]. There is a drop of 5.3 kcal/mol in the first step which is catalyzed by phosphofructokinase (PFK), and of 9 kcal/mol in the final step which involves pyruvate kinase (PK). All other ΔG's are comparable to RT. It should be mentioned that there is an uncertainty in the ΔG between GAP and 3PG since the NAD^+/NADH ratio has only been guessed to be 240; if instead that ratio were 20, say, the points for 3PG through PYR would have to be lowered by 3 kcal/mol. This remark might be relevant in view of the phase-shift analysis to be discussed below.

(ii) The other line of evidence concerning the profile of free energy changes comes from investigations of glycolytic oscillations [13,14]. Both intact yeast cells and cell-free extracts have oscillatory kinetics in a range of throughput [15] which corresponds to physiological conditions. The amplitudes of these oscillations are very large, with a typical ratio of 3 between maximum and minimum concentrations. Important information can be obtained from the phase shift between successive intermediates. Obviously, a phase determination is possible only when all intermediates undergo similar oscillations, which in the case of glycolysis happen to be nearly harmonic. Most of the phase lags between successive intermediates are close to zero, except for three: FDP lags behind F6P by approximately 225°, or $5\pi/4$ [4]; the PK reaction which is the last of the sequence induces a phase shift of 180°, or π; and in addition there is a phase difference between GAP and 3PG which depends on the details of the experimental conditions such as the ATP/ADP and NAD/NADH steady state ratios, and has been reported to be in the range 90° ± 30°, or $\frac{1}{2}\pi(1 \pm \frac{1}{3})$. These findings have been interpreted on the grounds of an empirical statement sometimes called the crossover theorem [3, 13,16]. It identifies as control sites of a reaction sequence those enzymes which are associated with large phase shifts. Hence the enzymes PFK, PK, and GAPDH are likely associated with reaction mechanisms leading to oscillations [17,18].

Further arguments which relate these phase shifts to the free energy profile have recently been given by Durup [19]. The point is that according to elementary kinetics, the free energy change ΔG_i in the ith step of a reaction chain, is connected to the forward and backward rates v_i, $\bar{v}_i$ by the relation $\Delta G_i = -RT \ln v_i/\bar{v}_i$. In a steady state with constant turnover $v = v_i - \bar{v}_i$, this implies that the rates are slowest, $v_i \approx v$, for the most irreversible steps, $-\Delta G_i \gg RT$, and they are fastest, $v_i \gg v$, for steps which are near equilibrium, $-\Delta G_i \lesssim RT$. Now, in an oscillatory mode, a nonzero phase shift can be sustained only across the slow steps, and hence requires an appreciable ΔG_i, while in a fast reaction both reactants and products oscillate in phase. (Note, however, that phase shifts of 180° may be trivially enforced by conservation laws, as for instance in the case [NAD] + [NADH] = const.). In this manner, the phase shifts observed in the PFK, PK, and GAPDH reactions are indicative of slow rates and thus of Gibbs free energy differences larger than RT. The argument is only qualitative in that it does not say anything about the step size; on the other hand, the phase determinations do not require absolute concentration measurements and therefore might be more reliable.

The combined evidence of (i) and (ii) suggests a free energy profile as shown schematically in fig. 2. The smallness of the free energy change in the central step between GAP and 3PG corresponds to a phase shift which is very sensitive to changes in the overall flux, or in the reactant concentrations.

Several aspects of this design are easily seen to be

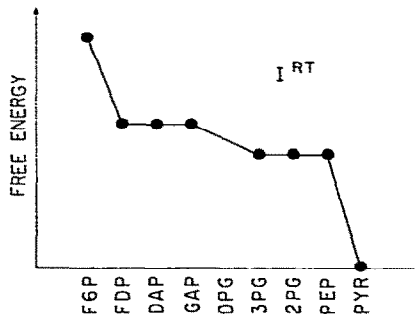

Fig. 2. Schematic view of the free energy profile in glycolysis. The two most irreversible steps at top and bottom are catalyzed by phosphofructokinase (PFK) and pyruvate kinase (PK) respectively. Phase shift analysis suggests a moderately irreversible step at the center. All three jumps are coupled to the adenosine phosphate pool: the PFK reaction converts ATP into ADP, the other two reactions produce ATP.

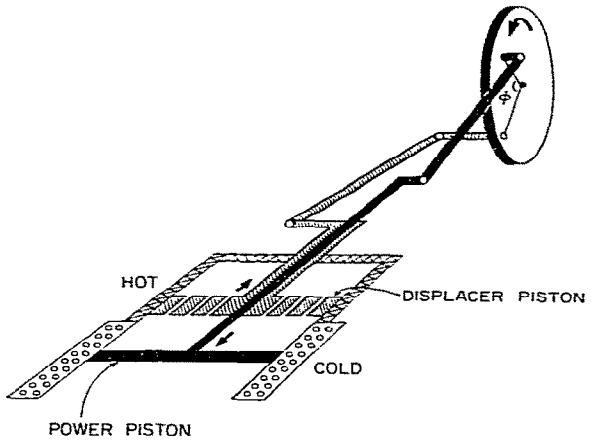

Fig. 3. Scheme of a Stirling motor. The rotation of the wheel (which stands here for the crankshaft) couples the oscillations of the displacer piston, i.e. of the thermal input, to those of the power piston, which takes up the mechanical output. The phase lag φ between input and output oscillations is indicated on the wheel.

beneficial for regulation purposes other than the generation of oscillations. These include: (i) A slow reaction at the beginning lends itself for effective control: a signal given at this point propagates rapidly down the chain, for instance to turn the subsequent reactions on or off. (ii) A highly irreversible step at the end helps to keep glycolysis disentangled from gluconeogenesis, in line with the general tendency of metabolism to separate synthetic from degradative pathways [1]. (iii) The enzyme PFK is activated by a low ATP/ADP ratio and inhibited by an abundance of ATP. This important control feature ensures that energy is generated only when there is need for it.

But on the other hand, there are certain design features which seem to be indicating that there is more to the oscillations than merely a byproduct role. (i) ATP, the inhibitor of PFK, is also a substrate of this enzyme reaction, and the activator ADP also a product. It is straightforward to see [20–22] that such enzyme properties bring on oscillatory behavior under non-equilibrium conditions. (ii) Pyruvate kinase is an allosteric enzyme which seems to have a tendency of its own towards oscillatory behavior [23–25]. It is hard to avoid the feeling that there must have been some purposes for PFK and PK to develop those rather elaborate properties which are so closely linked to the generation of oscillations, and the present analysis suggests purposes related to efficient energy conversion.

The subsequent sections are organized as follows. We start with a discussion of analogies, as well as differences, to heat engines and their performance (sect. 2). In sect. 3 we analyze the efficiency of a prototype chemical machine and its dependence on frequency, phase shifts, and other relevant parameters. Special attention is given to what we call "irreversible elasticity" in the response behavior of a chemical system, as it gives rise to a resonance where the efficiency may have a relative maximum. The results are then applied, in sect. 4, to discuss in some detail the particular design of the glycolytic system.

2. The heat engine analogy

The glycolysis machine converts energy from one form (sugar) into another (ATP). This suggests a comparison with the heat engine, which transforms thermal energy into mechanical work, in an oscillatory way. Though not complete in certain important aspects, the analogy turns out to be helpful in thinking about the design and efficiency of the machine. (An electrical net-

work analogy is given by the passage of a direct current with an a.c. component through a filter.)

Let us begin with the design of the systems, for which there are three common elements: (i) an oscillophor on either the input or output side, (ii) transmission of the oscillation to the other side, and (iii) regulation of the relative phase. In a heat engine, as for example the Stirling motor shown in fig. 3, this scheme is realized by (i) a rotating wheel or shaft on the mechanical (output) side, (ii) transmission of this cycling to the thermal input side via the displacer piston (or a system of valves in the case of internal combustion engines), and (iii) fixed phase setting built into the crankshaft (or the system of valves as well as the timing of the ignition spark). We shall argue in sect. 4 that the same elements exist in the design of the glycolysis machine.

In all the following, the discussion is restricted to harmonic variations. This is a reasonable approximation for most but not all [26] observed glycolytic oscillations. The harmonicity assumption implies a number of simplifications as to the dynamics of the system and usually requires small deviations (linear response) from the reference point of operation (which may be an equilibrium state or more generally, a stationary state). For harmonic oscillations the maximum attainable efficiency is reduced below that of Carnot-type cycles (typically by a factor $\pi/4$). On the other hand, as we have demonstrated previously [27], the analysis becomes very transparent and allows assessment of the effects of variation of characteristic parameters. A major benefit of the use of linear response theory is the separation of *internal* dynamical properties, as represented by the response function, from *external* features of the operation, such as the amplitudes of the driving forces, their frequency of oscillation and the relative phases. Our interest is in the effects of oscillations on the efficiency, and in possible maxima of the efficiency at nonzero frequencies. Based on our earlier work on the analysis of heat engines [27], we expect that such maxima require a kind of resonance between the driving oscillations (generated by PFK) and the internal dynamics (essentially the PK reaction).

Consider the following prototype chemical energy conversion machine [18]

$$\mathrm{A} \underset{k_0}{\overset{k_0}{\rightleftharpoons}} \mathrm{X}_1 \rightleftharpoons \mathrm{X}_2 \cdots \rightleftharpoons \mathrm{X}_n \underset{\bar{k}_n}{\overset{k_n}{\rightleftharpoons}} \mathrm{B} \qquad (1)$$

which could be either the entire glycolysis reaction mechanism or any part thereof. The symbols A and B represent reservoirs connected to the system $(\mathrm{X}_1, \ldots, \mathrm{X}_n)$, and the internal reactions leading from X_1 to X_n may be arbitrarily nonlinear. We assume $\mu_{\mathrm{A}}^{\mathrm{s}} \geqslant \mu_{\mathrm{B}}^{\mathrm{s}}$ for the chemical potentials in a reference steady state, which is either equilibrium ($\mu_{\mathrm{A}}^{\mathrm{s}} = \mu_{\mathrm{B}}^{\mathrm{s}}$) or subject to a steady net flux from A to B. Inasmuch as the material flux carries chemical energy, we call A the energy source and B the energy acceptor. We shall say that the system (1) converts chemical energy from form A to form B. (For glycolysis, the symbol A represents several reactants; the chemical energy is mainly contained in the sugar. B stands for various products, and part of the chemical energy is now in the form of ATP.) The exchange between the reservoirs and the system is assumed to be simply described in terms of the fluxes

$$j_{\mathrm{A}} = k_0 \mathrm{A} - \bar{k}_0 \mathrm{X}_1, \qquad j_{\mathrm{B}} = k_n \mathrm{X}_n - \bar{k}_n \mathrm{B}, \qquad (2)$$

with $j_{\mathrm{A}} = j_{\mathrm{B}} \equiv j^{\mathrm{s}}$ in a steady state. (Note the sign convention that j_{A} leaves reservoir A and j_{B} enters B.) The Gibbs free energy change in A, per unit time, is $\mu_{\mathrm{A}} j_{\mathrm{A}}$, and the amount $\mu_{\mathrm{B}} j_{\mathrm{B}}$ is transferred to B. If the reaction (1) is run cyclically with a period of $2\pi/\omega$ (either due to inherent oscillations or due to external periodic variations), then the average chemical power fluxes from A, and into B, are

$$P_{\mathrm{A}} = \frac{\omega}{2\pi} \oint \mu_{\mathrm{A}}(t) j_{\mathrm{A}}(t)\, \mathrm{d}t, \qquad P_{\mathrm{B}} = \frac{\omega}{2\pi} \oint \mu_{\mathrm{B}}(t) j_{\mathrm{B}}(t)\, \mathrm{d}t \qquad (3)$$

respectively. The difference

$$\Delta_{\mathrm{AB}} \equiv P_{\mathrm{A}} - P_{\mathrm{B}} \qquad (4)$$

constitutes dissipation and must be positive according to the second law.

The quantities analogous to P_{A} and P_{B} in the heat engine [27] are, respectively, the availability flux offered by the heat reservoir $(\omega/2\pi T) \oint \delta T \delta \dot{Q}\, \mathrm{d}t$, and the mechanically used power $(\omega/2\pi) \oint \delta p\, \delta \dot{V}\, \mathrm{d}t$. Again their difference represents the dissipative losses associated with the operation of the engine. The chemical machine and the heat engine differ, however, in several important respects. First, the engine revolves around a reference state which is usually taken to be an equilibrium state, whereas our chemical oscillations are, in general, superimposed on a steady state in which

there is throughput and dissipation:

$$P_A^s = \mu_A^s j^s, \quad P_B^s = \mu_B^s j^s, \quad \Delta_{AB}^s = (\mu_A^s - \mu_B^s) j^s. \tag{5}$$

A second distinction between a heat engine and our chemical machine is that equilibrium has two degrees of freedom in the former (p and T can be varied independently) but only one in the latter (where equilibrium requires $\mu_A = \mu_B$). This implies, thirdly, that the behavior in the quasistatic limit of periodic operation ($\omega \to 0$) is different in the two cases. There is no dissipation in that limit, in the heat engine, whereas the chemical machine shows a change with respect to the steady state. We show in the appendix that dissipation is minimized, for $\omega \to 0$, when $\delta\mu_A(t), \delta\mu_B(t)$ and therefore also $\delta j_A(t) = \delta j_B(t)$ all oscillate with equal phases. Denoting their respective amplitudes by $\delta\tilde{\mu}_A, \delta\tilde{\mu}_B, \delta_j$, we get the dissipation

$$\Delta_{AB}(\omega = 0) = \Delta_{AB}^s + \tfrac{1}{2}(\delta\tilde{\mu}_A - \delta\tilde{\mu}_B)\delta j. \tag{6}$$

At equilibrium where $\delta j \propto (\delta\tilde{\mu}_A - \delta\tilde{\mu}_B)$ the increment $o\Delta_{AB}(0) = \Delta_{AB}(\omega = 0) - \Delta_{AB}^s$ is always positive as it should be from the second law. But far from equilibrium, $\mu_A^s \gg \mu_B^s$, the current amplitude behaves more nearly as $\delta j \propto \delta\tilde{\mu}_A$, and $\delta\Delta_{AB}(0)$ may have either sign, depending on the relative sizes of $\delta\tilde{\mu}_A$ and $\delta\tilde{\mu}_B$. It appears therefore that dissipation can be reduced trivially by having the acceptor pool B oscillate more vigorously than the source A, $\delta\tilde{\mu}_B > \delta\tilde{\mu}_A$. Although this is correct, it does not provide an argument in favor of the evolutionary development of oscillations because twice the gain of (6) would be obtained by simply shifting the steady state μ_A^s, μ_B^s, j^s to $\mu_A^s + \delta\tilde{\mu}_A$, $\mu_B^s + \delta\tilde{\mu}_B, j^s + \delta j$. Therefore, in order to assess the benefit of imposed oscillations for a reduction of dissipation, the more meaningful comparison is between finite ω and $\omega = 0$. When a system cannot operate better at finite frequency that at $\omega = 0$ there is no point in using oscillations. If, on the other hand, the dissipation can be reduced below its value at $\omega = 0$, then it is beneficial to operate in the appropriate frequency range. As a quantitative measure for this gain we choose the relative reduction in dissipation

$$\epsilon(\omega) = \frac{\delta\Delta_{AB}(0) - \delta\Delta_{AB}(\omega)}{\Delta_{AB}(0)}. \tag{7}$$

In the following section we shall analyze this number for various systems trying to find conditions for it to be positive.

3. The concept and role of irreversible elasticity in increasing the efficiency

We saw in the introduction that the Gibbs free energy changes in the various parts of the glycolysis mechanism divide into three groups: the first is associated with the PFK reaction which initiates oscillations; the second with the GAPDH reaction which provides crucial phase shifts; and the third with the PK reaction which likely may have an oscillatory response. In this section we study simple models for the PK reaction and show that the presence of an oscillatory response, which we call irreversible elasticity, leads to an increase in overall free energy conversion.

First, we calculate the quantities $P_{A,B}$ and $\epsilon(\omega)$ for a chemical machine of type (1) with $n = 2$. For the moment we do not care how the oscillations in A and B originate; we simply assume that the machine is driven by

$$A = A^s + a\cos\omega t, \quad B = B^s + b\cos(\omega t - \varphi), \tag{8}$$

with given amplitudes a, b, frequency ω, and phase shift φ. Furthermore, we are not interested in the detailed nonlinear kinetics that may characterize the system. Rather we confine ourselves to the linear neighborhood of the steady state where we can write

$$\begin{pmatrix} \delta\dot{X}_1 \\ \delta\dot{X}_2 \end{pmatrix} + \mathbf{A}\begin{pmatrix} \delta X_1 \\ \delta X_2 \end{pmatrix} = \begin{pmatrix} k_0 a\cos\omega t \\ k_2 b\cos(\omega t - \varphi) \end{pmatrix} \tag{9}$$

with an arbitrary relaxation matrix $\mathbf{A}$. It is straightforward to derive the periodic solution of these rate equations:

$$\delta X_1(t) = \frac{1}{\lambda^2\sqrt{\Delta(\omega)}}\left(\sqrt{\Lambda_{22}^2 + \omega^2}\,k_0 a\cos(\omega t + \alpha_2 - \psi)\right.$$
$$\left. - \Lambda_{12}\bar{k}_2 b\cos(\omega t - \varphi - \psi)\right),$$

$$\delta X_2(t) = \frac{1}{\lambda^2\sqrt{\Delta(\omega)}}\left(-\Lambda_{21}k_0 a\cos(\omega t - \psi)\right.$$
$$\left. + \sqrt{\Lambda_{11}^2 + \omega^2}\,\bar{k}_2 b\cos(\omega t - \varphi + \alpha_1 - \psi)\right). \tag{10}$$

Here we have introduced a number of characteristic quantities: with

$$\lambda^2 \equiv \det\mathbf{A}, \quad s \equiv \mathrm{Tr}\,\mathbf{A} \tag{11}$$

the resonance denominator $\Delta(\omega)$ reads

$$\Delta(\omega) \equiv (1 - \omega^2/\lambda^2)^2 + \omega^2 s^2/\lambda^4; \tag{12}$$

the intrinsic phase shift ψ,

$$\cos\psi = \frac{1 - \omega^2/\lambda^2}{\sqrt{\Delta(\omega)}}, \quad \sin\psi = \frac{\omega s/\lambda^2}{\sqrt{\Delta(\omega)}}, \tag{13}$$

indicates how much δX_2 lags behind δA in case $b = 0$ (i.e. for driving oscillations in A only), or how much δX_1 lags behind δB in case $a = 0$ (i.e. for only B driving the system). With ω increasing from 0 towards ∞, ψ grows from 0 to π; at $\omega = \lambda$ we have $\psi = \frac{1}{2}\pi$. The other two relative phases $\alpha_{1,2}$ are defined as

$$\cos\alpha_i = \Lambda_{ii}/\sqrt{\Lambda_{ii}^2 + \omega^2}, \quad \sin\alpha_i = \omega/\sqrt{\Lambda_{ii}^2 + \omega^2}, \tag{14}$$

and they grow from $\alpha_1 = 0$ at $\omega = 0$ to $\alpha_i = \frac{1}{2}\pi$ as $\omega \to \infty$. The quantity α_1 is the phase lag of δX_1 with respect to δX_2 in a response to δB only, and α_2 determines how much δX_2 lags behind δX_1 in a response to δA.

Using the result (10) and assuming ideality for the reservoirs, $\delta\mu_A = k_B T \delta A/A$, $\delta\mu_B = k_B T \delta B/B$, we can easily evaluate the integrals (3) for $P_{A,B}$, or for $\delta P_{A,B} \equiv P_{A,B} - P^s_{A,B}$. In terms of the fluxes

$$j_A^+ \equiv k_0 A^s, \quad j_B^- \equiv \bar{k}_2 B^s, \tag{15}$$

and of the dimensionless quantities $\delta p_{A,B}$,

$$\delta p_{A,B} = \frac{A^s B^s}{ab} \frac{2/k_B T}{\sqrt{j_A^+ j_B^-}} \delta P_{A,B}, \tag{16}$$

the result reads

$$\delta p_A = R\left(1 - \frac{\bar{k}_0\sqrt{\Lambda_{22}^2 + \omega^2}}{\lambda^2\sqrt{\Delta(\omega)}}\cos(\psi - \alpha_2)\right) + \left(\frac{j_B^-}{j_A^+}\right)^{1/2} \frac{\bar{k}_0\Lambda_{12}}{\lambda^2\sqrt{\Delta(\omega)}}\cos(\psi + \varphi),$$

$$\delta p_B = -\left(\frac{j_A^+}{j_B^-}\right)^{1/2} \frac{k_2\Lambda_{21}}{\lambda^2\sqrt{\Delta(\omega)}}\cos(\psi - \varphi) - \frac{1}{R}\left(1 - \frac{k_2\sqrt{\Lambda_{11}^2 + \omega^2}}{\lambda^2\sqrt{\Delta(\omega)}}\cos(\psi - \alpha_1)\right), \tag{17}$$

where R is a parameter related to the ratio of the two amplitudes a and b,

$$R \equiv \frac{a}{A^s}\frac{B^s}{b}\left(\frac{j_A^+}{j_B^-}\right)^{1/2}. \tag{18}$$

The term proportional to R in δp_A is always positive, and thus adds to the input. It tends to decrease the efficiency according to (7) which might suggest that one chooses a small R. But then the term $\sim 1/R$ in δp_B is always negative, subtracting from the output and again hampering the efficiency. In fact, these two terms represent the unavoidable dissipative losses associated with the oscillations of A and B. We expect their combined influence to be minimized for R of the order of 1.

The interesting terms in (17) are the coupling terms proportional to Λ_{12} and Λ_{21}. They depend on the phase shift φ and may be adjusted so as to improve the efficiency. We thus minimize the dissipation Δ_{AB} (which is proportional to the overall entropy production) with respect to φ and obtain the condition

$$\tan\varphi = \frac{j_A^+ k_2\Lambda_{21} - j_B^-\bar{k}_0\Lambda_{12}}{j_A^+ k_2\Lambda_{21} + j_B^-\bar{k}_0\Lambda_{12}}\tan\psi. \tag{19}$$

This formula has some interesting implications. Consider first the case of a simple monomolecular reaction inside the machine,

$$X_1 \underset{\bar{k}_1}{\overset{k_1}{\rightleftharpoons}} X_2. \tag{20}$$

Then $\Lambda_{21} = -k_1$ and $\Lambda_{12} = -\bar{k}_1$, and (19) can be written as

$$\tan\varphi = \frac{j^+ - j^-}{j^+ + j^-}\tan\psi \tag{21}$$

where

$$j^+ = k_0 k_1 k_2 A^s/\lambda^2, \quad j^- = \bar{k}_0\bar{k}_1\bar{k}_2 B^s/\lambda^2, \tag{22}$$

are the overall forward and backward fluxes, respectively. Near equilibrium we have $j^+ \approx j^-$ and thus $|\tan\varphi| \ll |\tan\psi|$. This implies that the dissipation is smallest for $\varphi \approx 0$, at low frequencies $\omega < \lambda$, and for $\varphi \approx \pi$ at high frequencies $\omega > \lambda$. Far from equilibrium, on the other hand, we have $j^- \ll j^+$ and therefore $\varphi \approx \psi$ for the optimal phase. In case the reaction

system is far from equilibrium and more complicated than (20), then the rates Λ_{12} and Λ_{21} may have a different sign. The denominator in (19) may then go to zero and the optimum choice for φ is $+\pi/2$ or $-\pi/2$. We suggest that this is the case in the last step of the glycolytic reaction mechanism.

Evaluation of the results (17) for very slow ($\omega \to 0$) and very fast ($\omega \to \infty$) imposed oscillations is given in the Appendix.

Next we analyze the frequency dependence of the expressions (17). Since the goal is to apply the result to the lower part of the glycolytic chain, with $X_1 \rightleftharpoons X_2$ symbolizing the pyruvate kinase reaction, we assume that there is a large drop in chemical potential within our system,

$$\mu_1 - \mu_2 \equiv \delta \gg k_B T. \tag{23}$$

As to the coupling with the external baths A, B we assume that the reactions $A \rightleftharpoons X_1$ and $X_2 \rightleftharpoons B$ are nearly equilibrated. Then the ω-spectra of δp_A, δp_B, and the efficiency $\epsilon(\omega)$ are mainly determined by the characteristics of the $X_1 \to X_2$ conversion. We shall see that the dissipative aspects of that conversion tend to reduce $\epsilon(\omega)$ below zero whereas there is a possibility of positive $\epsilon(\omega)$ in case the reaction bears a tendency towards oscillations.

Consider first the linear system (20) in which the dissipative influence dominates. The steady state concentrations, in keeping with the above assumptions, are

$$X_1^s = K_0 A^s, \quad X_2^s = K_1 \exp(-\delta/k_B T) X_1^s,$$

$$B^s = K_2 X_2^s, \tag{24}$$

where $K_i \equiv k_i/\bar{k}_i$ are the respective equilibrium constants, see (1). The number

$$K \equiv K_1 \exp(-\delta/k_B T) \tag{25}$$

is an interesting parameter of the system. $K \approx 1$ means that the steady state concentrations of X_1 and X_2 are about equal (whereas equilibrium would require $X_2 \gg X_1$ because of $\exp(\delta/k_B T) \gg 1$. For the overall fluxes $j^{\pm}$, see (21), our assumptions imply

$$j^+ - j^- \approx j^+ = \gamma k_0 A^s, \quad \gamma \ll 1. \tag{26}$$

Therefore the minimum dissipation condition for the relative phase implies $\varphi = \psi$. From the condition $\gamma \ll 1$ it also follows that $j_A^+ \approx j_B^-$, see (15), and thus

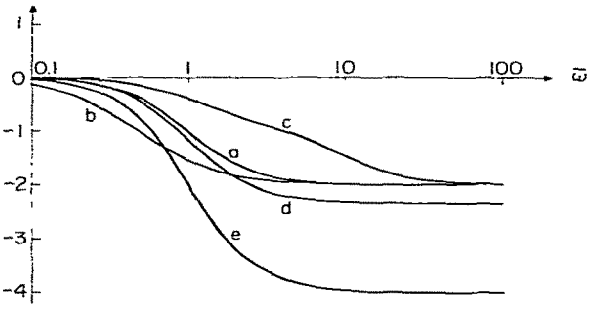

Fig. 4. Frequency spectrum of the incremental efficiency for the purely dissipative system (20). The curves show $\Delta_{AB}(0) - \Delta_{AB}(\bar{\omega})$, which is proportional to the efficiency $\epsilon(\omega)$ defined in (7). For all choices of parameters, and all frequencies, $\epsilon(\omega)$ is negative which implies that there is no benefit in driving such a system in an oscillatory way. The five curves correspond to the following choices of parameters. a: $K = 1$, $R = 1$ or 2; b: $K = 3$, $R = 1$; c: $K = 0.1$, $R = 1$; d: $K = 1$, $R = 3$; e: $K = 1$, $R = 0.3$.

$$R = \frac{a}{A^s} \Big/ \frac{b}{B^s} = \frac{\delta\bar{\mu}_A}{\delta\bar{\mu}_B}. \tag{27}$$

The three parameters R, γ, and K are the important characteristics of this linear system. Neglecting wherever possible γ and $\exp(-\delta/k_B T)$ as compared to 1, we find that the formulae (17) reduce to

$$\delta p_A = R \frac{\gamma + \bar{\omega}^2}{1 + \bar{\omega}^2},$$

$$\delta p_B = \frac{\gamma}{((1+\bar{\omega}^2)(1+(K\bar{\omega})^2)^{1/2}} - \frac{1}{R} \frac{(K\bar{\omega})^2}{1+(K\bar{\omega})^2}. \tag{28}$$

where $\bar{\omega} \equiv \omega/\bar{k}_0$ is a conveniently reduced frequency. Fig. 4 illustrates these results for various choices of parameters. The dissipation associated with the oscillations, $\delta\Delta_{AB}(\omega) \equiv \delta p_A(\omega) - \delta p_B(\omega)$, is monotonically increasing with ω which means that the efficiency (7) is always negative. Thus the system cannot do better at non-zero frequencies than it does in a quasi-static operation.

The situation is different in a system which possesses what we shall call "irreversible elasticity", that is the system has a reaction mechanism such that con-

centration oscillations are possible. In general, the relaxation matrix $\mathbf{A}$ of eq. (9) can be decomposed as follows,

$$\mathbf{A} = \mathbf{L} \cdot \mathbf{\Gamma}^{-1}, \tag{29}$$

$\Gamma_{ij} \equiv \langle \delta X_i \delta X_j \rangle$ being the static correlations of fluctuations, and $\mathbf{L}$ the analogue of Onsager's transport matrix [28]. At equilibrium, $\mathbf{L}$ has to be symmetric which prevents $\mathbf{A}$ from having complex eigenvalues. However, far from equilibrium where time reversal symmetry is broken, an antisymmetric part $\mathbf{A} = \frac{1}{2}(\mathbf{L} - \mathbf{L}^{\mathrm{T}})$ may appear. This causes relaxation towards the stationary state to be oscillatory if $|A_{12}|$ is large enough to ensure

$$\sqrt{\det \mathbf{A}} \equiv \lambda > \tfrac{1}{2} s \equiv \tfrac{1}{2} \operatorname{Tr} \mathbf{A}. \tag{30}$$

$\mathbf{A}$ has been called "irreversible circulation" by Tomita and Tomita [29]. From the point of view of response theory it represents an elasticity [30]. Thus in the present context we use the term "irreversible elasticity".

Because of the strong irreversibility condition (23) our chemical machine may well have a non-zero $\mathbf{A}$. In fact, it is easy to show that for the above monomolecular reaction system $\mathbf{A} = \frac{1}{2}(j^{+} - j^{-})\begin{pmatrix} 0 & 1 \\ -1 & 0 \end{pmatrix}$. This asymmetry is, however, not sufficient to ensure (30). Appropriate nonlinearities must be invoked in the reaction mechanism in order to get this condition fulfilled. Knowing that there are many models of such systems, let us discuss a typical case. We take the $\mathbf{A}$ matrix corresponding to the previous example, for $\gamma \to 0$, which is

$$\bar{k}_0 \begin{pmatrix} 1 & 0 \\ 0 & 1/K \end{pmatrix} \tag{31}$$

and add-off-diagonal terms as follows:

$$\mathbf{A} = \bar{k}_0 \begin{pmatrix} 1 & \nu/K \\ -\nu & 1/K \end{pmatrix}. \tag{32}$$

This corresponds to an irreversible elasticity

$$A = \nu \bar{k}_0 X_1^{s} \begin{pmatrix} 0 & 1 \\ -1 & 0 \end{pmatrix} \tag{33}$$

if we assume Poissonian behavior for the correlations.

From (32) we find the ratio

$$s/\lambda = \frac{\sqrt{K} + 1/\sqrt{K}}{\sqrt{1 + \nu^2}} \tag{34}$$

which is of the order of 1 or less, and in turn implies oscillatory relaxation if

$$\nu > \sqrt{K} + 1/\sqrt{K}. \tag{35}$$

Thus the condition for irreversible elasticity is weakest in the case $K = 1$, or $X_1^{s} = X_2^{s}$, where evaluation of leads to

$$\delta p_{\mathrm{A}} = R\left(1 - \frac{1 + \nu^2 + \bar{\omega}^2}{\Delta_\nu(\bar{\omega})}\right) - \frac{\nu}{\sqrt{\Delta_\nu(\bar{\omega})}} \frac{(1 + \nu^2 - \bar{\omega}^2)^2 - 4\bar{\omega}^2}{\Delta_\nu(\bar{\omega})}$$

$$\delta p_{\mathrm{B}} = \frac{\nu}{\sqrt{\Delta_\nu(\bar{\omega})}} - \frac{1}{R}\left(1 - \frac{1 + \nu^2 + \bar{\omega}^2}{\Delta_\nu(\bar{\omega})}\right) \tag{36}$$

with the resonance denominator $\Delta_\nu(\bar{\omega}) = (1 + \nu^2 - \bar{\omega}^2)^2 + 4\bar{\omega}^2$.

A few typical spectra are shown in figs. 5 and 6. For $\nu \leqslant 2$ the picture resembles the purely dissipative case of fig. 4, in agreement with condition (35). At larger irreversible elasticity, however, there is a marked difference: the curves exhibit resonance behavior near $\bar{\omega} = \lambda/\bar{k}_0 = \sqrt{1 + \nu^2}$ where $\delta p_{\mathrm{A}} \approx \frac{1}{2}(R + 1)$ and $\delta p_{\mathrm{B}} \approx \frac{1}{2}(1 - 1/R)$. Outside the resonance region there is no advantage in an oscillatory operation; the increment δp_{B} to the output is in fact negative. But in a narrow range around $\bar{\omega} = \lambda/\bar{k}_0$ where the phase shift between X_1 and X_2 is close to $\pi/2$, the extra input δp_{A} is smaller than at zero frequency and the output δp_{B} positive. This is the interesting feature of irreversible elasticity; it opens a chance for improved performance in a certain range of frequencies of oscillation.

To sum up, when a chemical machine with sufficient irreversible elasticity is driven at resonance frequency, the increased net input δP_{A} may be converted into an additional output δP_{B} which affects the efficiency in a favorable way. For this to happen the phase relation between the oscillations in A and B must be carefully regulated so that, within a cycle, the flux into B is above average when the chemical potential $\mu_{\mathrm{B}}(t)$ is at its maximum, and below average at times of low μ_{B}.

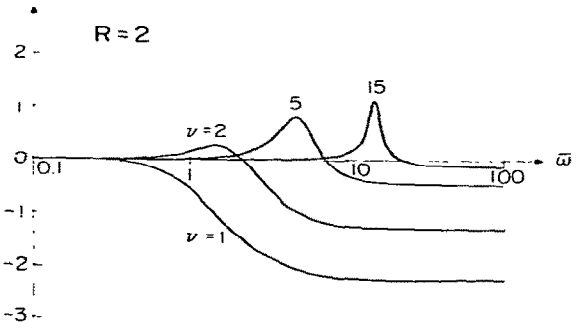

Fig. 5. Incremental efficiency for the system (32) which possesses irreversible elasticity. The curves show $\Delta_{AB}(0) - \Delta_{AB}(\omega)$ according to eq. (36). For $\nu \lesssim 2$ the behavior is similar to that of a purely dissipative system, see fig. 4. But for $\nu \gtrsim 2$ the system has a resonance frequence near $\bar{\omega} = \nu$, and the efficiency becomes enhanced over the static value ($\omega = 0$). Parameters are $R = 2$, and ν as indicated.

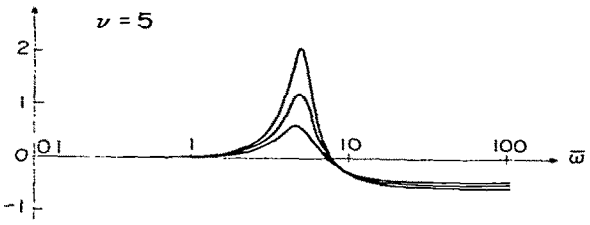

Fig. 6. Same as fig. 5 with parameters $\nu = 5$ and $R = 1, 3, 5$ in order of increasing peak height.

4. The glycolysis machine

The results of the preceding sections are now applied to the analysis of the glycolytic oscillations from the point of view of efficiency [31]. Consider again the sequence of steps shown in fig. 2. We assume that there is a steady supply of F6P which is then converted into FDP in the phosphofructokinase reaction

$$\text{F6P} + \text{ATP} \xrightarrow[\text{PFK}]{} \text{FDP} + \text{ADP}. \qquad (37)$$

There is agreement that this is the basic autonomous oscillator in the glycolytic chain, and its mechanism is well understood [20,21,32,33]. The oscillations generated here propagate down the chain without appreciable phase delay because of near equilibrium conditions between FDP through GAP. The phase shift acquired in the GAPDH/PGK reaction is indicative of some slowing down at this step, or equivalently, of a drop in free energy. The two subsequent steps are again fast, with quasi-equilibrium holding between 3PG through PEP, and then there is the final reaction which involves the largest free energy drop of all:

$$\text{PEP} + \text{ADP} \xrightarrow[\text{PK}]{} \text{PYR} + \text{ATP}. \qquad (38)$$

We suggest that this last step is a driven oscillator whose intrinsic irreversible elasticity enables the system to work with higher efficiency than it would without any oscillations, given the same steady state values of the chemical potentials.

What is the evidence for the presence of irreversible elasticity in this reaction? The kinetics of the PK reaction is in fact rather complicated. First the enzyme activity shows some cooperativity with respect to the substrate PEP, the Hill coefficient being close to 2 [24]. Secondly, PK is activated, and the PEP-cooperativity removed, by FDP which is a product of the PFK reaction [24,25]. Third, PK is inhibited by its own product ATP [25]. As shown by Dynnik and Selkov [23], the FDP activation alone can give rise to an oscillatory behavior in the lower part of the glycolytic chain. On the other hand, self-sustained oscillations have not been found experimentally. This may be due to a number of reasons, such as the PK reaction being run below marginal stability caused, for example, by product inhibition due to ATP counteracting the oscillatory tendency sufficiently strongly to prevent its spontaneous manifestation. The issue of oscillatory behavior in the PK reaction needs further investigation both theoretically and experimentally.

The rate equations for PEP and PYR, linearized around the steady state, would then read

$$\frac{\mathrm{d}}{\mathrm{d}t}\begin{pmatrix}\delta\,[\text{PEP}]\\ \delta\,[\text{PYR}]\end{pmatrix} + \mathbf{A}\begin{pmatrix}\delta\,[\text{PEP}]\\ \delta\,[\text{PYR}]\end{pmatrix} = \begin{pmatrix}a\cos\omega t\\ b\cos(\omega t - \varphi)\end{pmatrix} \qquad (39)$$

where the relaxation matrix $\mathbf{A}$ has complex eigenvalues and the right hand side represents driving forces that combine the effects of the oscillations of the direct reactants 2PG, ADP, ATP, as well as the periodic PK activity variations induced by oscillating FDP, PEP, and ATP. It appears beyond reach, with the available data, to assess the effective amplitudes a, b, and the

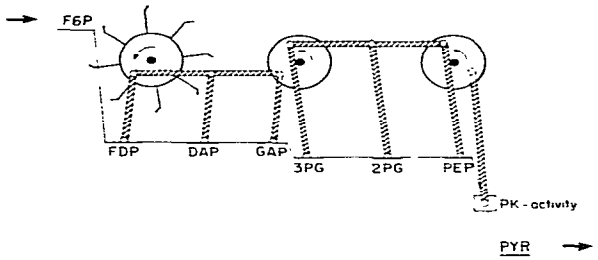

Fig. 7. Schematic view of the glycolysis machine. The free energy released in the conversion of F6P into FDP "turns the wheel" that forces all the subsequent intermediates to oscillate. There is a phase shift at the center and another one between the PEP oscillations and those of the pyruvate kinase activity. Oscillations of the adenosine phosphates, although important, are not shown. If the conversion of PEP + ADP into PYR + ATP possesses irreversible elasticity, and if there is resonance with the driving oscillations, the efficiency of free energy conversion is enhanced with respect to the non-oscillatory operation.

relative phase φ. Therefore, at this point, we can argue only qualitatively that if there is resonance between the driving frequency of the PFK reaction and the frequency associated with the PK reaction, then the oscillatory mode of operation is beneficial from the point of view of efficiency: for the same mean values of the chemical potentials, there is an increased output of chemical energy, per unit time, into the acceptor reservoir.

To estimate the order of magnitude of the effect note that the output enhancement near resonance, according to (36), is $\delta p_B \approx \frac{1}{2}(1 - 1/R)$, or

$$\delta P_B \approx \frac{a}{A^s}\,\frac{b}{B^s}\,\sqrt{j_A^+ j_B^-}\,\frac{k_B T}{4}\left(1 - \frac{1}{R}\right). \tag{40}$$

Compared to the dissipation between A and B which in our model case is $\Delta^s_{AB} = (\mu^s_A - \mu^s_B) j^s \approx \gamma k_0 A^s (\mu^s_A - \mu^s_B)$, we get

$$\delta P_{AB}/\Delta^s_B \approx \frac{a}{A^s}\,\frac{b}{B^s}\,\frac{k_B T}{\gamma(\mu^s_A - \mu^s_B)}\,\frac{1}{4}\left(1 - \frac{1}{R}\right). \tag{41}$$

$\gamma \ll 1$ is the ratio between the net flux j^+ and the incoming flux j^+_A (the limit $\gamma \to 1$ may not be taken in (41)). For large amplitude oscillations, $a/A^s \sim b/B^s \sim 1$, and taking $R \sim 2$, $\gamma(\mu^s_A - \mu^s_B)/k_B T \sim 1$, we can easily get some 10–20% for the ratio (41). When applied to the glycolytic system we see, however, that Δ^s_{AB} only represents the dissipation between the levels of PEP and PYR which is roughly one half of the total dissipation, see fig. 1. The overall improvement in efficiency, due to the postulated resonance effect, may thus be of the order of 5–10%. Since we are dealing with a vital process this may well have been relevant for evolution.

Our picture of the glycolysis machine can now be summarized as shown symbolically in fig. 7, and it is instructive to compare it to the mechanical engine [‡] shown in fig. 3. The common features are

(i) Generation of oscillations. In glycolysis this occurs at the input side whereas in heat engines the wheel or shaft is driven by the output power.

(ii) Transmission of the oscillations to the opposite side of the system. The mechanism by which this is done distinguishes the various types of machines from the point of view of engineering. It is very simple in the Stirling engine, fig. 3; internal combustion engines involve systems of valves and crank-shafts. The glycolysis machine, in addition to using oscillations of the direct reactants, in the PK reaction, employs periodically induced variation of the enzymatic activity.

(iii) Phase regulation between input and output side. In the example of fig. 3 this is done by fixing the angle φ on the wheel. In glycolysis the reactions leading from GAP to 3PG seem to play the role of a phase-shifter that adjust the phase depending on the ADP/ATP and NAD/NADH ratios.

Note that the adenosine phosphate system has not explicitly been included in fig. 7. Implicitly, however, it is thoroughly involved in all the three major steps.

Our conjecture involves a number of testable predictions. First, it should be possible to find out whether indeed the PK reaction, under the appropriate non-equilibrium conditions, shows oscillatory relaxation. Secondly, it is crucial to establish that the above-mentioned resonance holds. Third, even at resonance a gain in efficiency depends on several relations among the parameters to be fulfilled, such as $\nu \geqslant \sqrt{K} + 1/\sqrt{K}$ or $R \geqslant 1$. Before those relations can be tested, however, a more detailed identification of the param-

[‡] Some readers may prefer an electrical analogy where the dissipation represents the absorptive aspect of the system, i.e. the imaginary part of the refractive index. It might be interesting to speculate about the chemical analogue of the real part of the refractive index.

eters in terms of properties of the PK reaction is necessary.

Acknowledgements

We thank Professor B. Hess and Dr. Y. Termonia for stimulating discussions.

Appendix

The general result (7) is evaluated here for the low and high frequency limit, and for the simple reaction (20) describing the conversion of X_1 to X_2. The relaxation matrix **A** is

$$\begin{pmatrix} \bar{k}_0 + k_1 & -\bar{k}_1 \\ -k_1 & \bar{k}_0 + k_2 \end{pmatrix}. \tag{A.1}$$

Denoting the amplitudes of the chemical potential variations by $\delta\tilde{\mu}_A = k_B T a/A^s$ and $\delta\tilde{\mu}_B = k_B T b/B^s$, the unreduced quantities $\delta P_{A,B}$ in the limit $\omega \to 0$ are

$$\delta P_A = \tfrac{1}{2}\delta\tilde{\mu}_A(\delta j^+ - \delta j^- \cos\varphi),$$

$$\delta P_B = \tfrac{1}{2}\delta\tilde{\mu}_B(\delta j^+ \cos\varphi - \delta j^-), \tag{A.2}$$

with

$$\delta j^+ = k_0 k_1 k_2 a/\lambda^2, \quad \delta j^- = \bar{k}_0\bar{k}_1\bar{k}_2 b/\lambda^2. \tag{A.3}$$

The minimum dissipation condition $\varphi = 0$ (which in this case is also the condition for maximum output) leads to

$$\delta\Delta_{AB}(\omega = 0) = \tfrac{1}{2}(\delta\tilde{\mu}_A - \delta\tilde{\mu}_B)\delta j, \tag{A.4}$$

where $\delta j = \delta j^+ - \delta j^-$ is the amplitude of the net flux oscillation. This is the result quoted in eq. (6). For equilibrium where $k_0 k_1 k_2 A^s = \bar{k}_0\bar{k}_1\bar{k}_2 B^s$ we get

$$\delta\Delta_{AB}(\omega = 0) = \Delta_{AB}(\omega = 0) = \tfrac{1}{2}k_B T \frac{k_0 k_1 k_2 A^s}{\lambda^2}\left(\frac{a}{A^s} - \frac{b}{B^s}\right)^2 \tag{A.5}$$

which is positive in accordance with the second law.

For $\omega \to \infty$, the coupling terms in (17) vanish, and $\delta p_A = R$, $\delta p_B = -1/R$, or

$$\delta P_A = \tfrac{1}{2}\bar{k}_0 a\delta\tilde{\mu}_A, \quad \delta P_B = -\tfrac{1}{2}\bar{k}_2 b\delta\tilde{\mu}_B. \tag{A.6}$$

Clearly, oscillations of A, B that are fast compared to the machine's internal dynamics cannot generate any additional throughput but only add to the dissipation.

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