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19. Nonjunctional conductance decreases before pH_i changes when cell pairs are treated with any low pH solution, including membrane-impermeant strong acids. The pH_i at which the late rise in nonjunctional conductance occurs depends upon the rate of change of pH_i and might be mediated by a slower secondary process, such as an increase in cytoplasmic calcium.
20. In considering the significance of uncoupling assayed electrically, it should be noted that a coefficient for coupling of an inert tracer molecule (expressed as a concentration ratio) is determined by the ratio of permeabilities through junctional and nonjunctional membranes, rather than by the ratio of the electrical conductances of those membranes. If, as we believe, gap junction channels are closed by decreased pH_i in an all-or-none fashion, changes in junctional conductance are a good measure of changes in permeability to any (permeable) molecule. Electrical conductance of nonjunctional membranes may be due to a variety of channels that are specifically permeable to simple ions, and therefore changes in nonjunctional conductance or in electrical coupling coefficients are probably poor measures of changes in nonjunctional permeability or coupling coefficients for small molecules.
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Concentration Oscillations and Efficiency: Glycolysis

Abstract. *The oscillations observed in glycolysis are analyzed from the point of view of efficiency of free energy conversion. It is suggested that the mechanisms generating these oscillations may have evolved in order to reduce the dissipation of free energy.*

A number of biochemical reaction systems show periodic changes (oscillations) in the concentrations of their intermediates (*1*, *2*). The most extensively studied case of such metabolic oscillations is that of glycolysis, particularly in yeast cells and in cell-free extracts of yeast. Much effort has been devoted to determining the detailed mechanisms that lead to oscillatory behavior (*3*–*6*). Very little is known, however, about the reasons for the emergence of such mechanisms in the course of evolution. There is an argument that they are accidental by-products of regulatory features that are built into the system for the purpose of control. We suggest here another reason: oscillations may enhance the efficiency of free energy conversion from sugar to ATP (*7*). In an anaerobic environment, where glycolysis provides most of a cell's free energy supply, this may be a factor that favors an evolutionary development toward oscillatory operation.

Glycolysis is the degradation of sugars to pyruvate, which is further metabolized to alcohol (in yeast fermentation), lactic acid (in muscle), or acetyl coenzyme A (under aerobic conditions). Depending on the available source of free energy, which may be any of a variety of sugars or glycogen, there are different entries into the glycolytic pathway (*8*). Their point of convergence is the level of F6P, after which there are eight more reaction steps in the overall reaction

$$F6P + 2P_i + 3ADP + NAD^+ \rightarrow 2PYR + 3ATP + 2NADH + H^+$$

The overall drop in Gibbs free energy (ΔG) is about 14 kcal/mole. This does not mean, however, that in each of the eight steps the free energy decreases by ~ 1.8 kcal/mole, or $3RT$ (R is the gas constant and T is absolute temperature). Rather, the evidence points to a free energy profile with three distinct steps (Fig. 1). (The free energy profile takes full account of all reactants in each reaction step; however, we show only one identifying reactant on each level.)

The first reaction in the sequence, catalyzed by PFK, is highly irreversible (*9*, *10*). This is the primary oscillophor (generator of species oscillations) in the system, so that by necessity the reaction must proceed far from equilibrium. The relatively large Gibbs free energy change is attained in this step by conversion of ATP to ADP. Several models have been proposed that explain the occurrence of spontaneous oscillations on the basis of known regulatory features of PFK such as product activation, substrate inhibition, and allosteric properties (*3*–*5*).

The last reaction, which is catalyzed by PK, also takes place far from equilibrium; in fact, about half the total free energy loss occurs at this point. The enzymatic activity pattern of PK contains a number of features that make this reaction another likely candidate for being an oscillophor: there is activation by FDP, which alone could give rise to oscillations (*11*); furthermore, there is cooperativity with respect to the substrate PEP and inhibition by the product ATP (*12*, *13*). However, no experimental observations have been reported of independent oscillations in the lower part of the glycolytic pathway. These facts suggest the hypothesis, as yet unconfirmed, that the system is below marginal stability with respect to the onset of oscillations, but is in a regime where perturbations of the steady state decay in an oscillatory way. This provides the system with a resonance potentiality when driven by external oscillations—that is, those generated in the PFK reaction. We will see what effect this has on the efficiency of free energy throughput.

There is almost equilibrium among the intermediates FDP through PEP ($\Delta G \lesssim RT$) except for a drop in ΔG that probably occurs between GAP and 3PG. This step involves the allosteric enzyme GAPDH and couples glycolysis to the oxidation reaction ($NAD^+/NADH$).

Evidence for the free energy profile of Fig. 1 comes from concentration measurements (*14*, *15*) and from an analysis of phase relations in the oscillatory mode (*16*). Appreciable phase lags have been found only across the three steps mentioned—the PFK, PK, and GAPDH reactions. Hence these are slow steps compared to the others, which indicates that they are the farthest from equilibrium (*17*). The phase lag at the center (GAPDH) is very sensitive to changes in the steady-state ratios ATP/ADP and $NAD^+/NADH$, whereas the shifts across PFK and PK appear to be independent of such details.

From this partitioning of the total free energy decrease, we postulate the fol-

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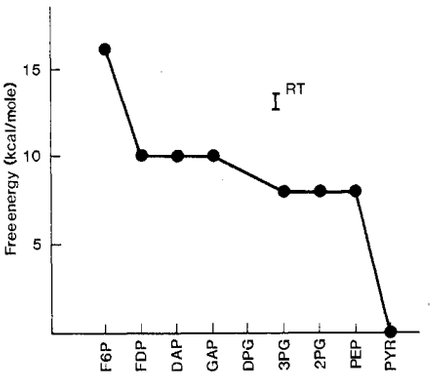

Fig. 1. Schematic view of the free energy profile in glycolysis. Reactions are nearly equilibrated, except those that are coupled to the adenosine phosphate pool: the PFK reaction at the top of the sequence, the GAPDH reaction at the center, and the PK reaction at the end. The thermal energy $RT \approx 0.6$ kcal/mole is shown for comparison.

lowing. Oscillations are generated in the first reaction step, at the expense of nearly half the available free energy. They propagate down the chain and drive all subsequent species into forced oscillations; in addition, the ATP/ADP subsystem transmits the oscillations directly to the other two crucial steps. All this is well substantiated experimentally. The role of the central step is postulated to be regulation of the phase on the input side of the PK reaction. This is important for the performance of the system since, depending on that phase, the PK reaction may either enhance or decrease the free energy throughput, as compared to steady-state operation (phase shifts from 15 ° to 120 ° have been reported). For an enhancement to occur, it is furthermore necessary that the intrinsic frequency of the PK response be close to resonance with the driving frequency.

To demonstrate the important point of regulation of dissipation near resonance, we calculated the dissipation of free energy in chemical reaction systems far from equilibrium for the case of oscillatory input and output (*18*). With reference to the lower end of the glycolytic pathway, consider the sequence

$$A \rightleftharpoons \text{PEP} \rightarrow \text{PYR} \rightleftharpoons B \qquad (1)$$

where the central portion symbolizes the highly irreversible PK reaction and A represents the free energy source (the oscillatory concentrations of the intermediates preceding PEP). In the PK reaction both pyruvate and ATP are produced. The concentrations in the adenylate pool oscillate in time as a result of the oscillations produced in PFK and consequent reactions. We represent this complex situation by the last step in Eq. 1. Both A and B are oscillating around steady-state values A_s and B_s at frequency ω and phase shift φ between A and B

$$A(t) = A_s + \delta A \cos \omega t$$
$$B(t) = B_s + \delta B \cos (\omega t - \varphi) \qquad (2)$$

We compare two basically different possibilities for the PK reaction. First, we assume purely dissipative behavior—that is, we take the relaxation of perturbations around the steady state to be of the exponential type. Second, we contrast this with the case where the PK reaction has an irreversible elasticity (*18*), which means that the relaxation into the steady state is oscillatory, with complex eigenfrequencies. This case requires the system to be far from equilibrium.

The free energy dissipated per unit time, on the average over a cycle of operation, is the difference between the net chemical power released by A and the power uptake by the reservoir B

$$\Delta(\omega) = \frac{\omega}{2\pi} \oint \mu_A(t)\ \mathcal{J}_A(t)\ dt - \frac{\omega}{2\pi} \oint \mu_B(t)\ \mathcal{J}_B(t)\ dt \qquad (3)$$

where μ_A and μ_B are the respective chemical potentials, $\mathcal{J}_A(t)$ is the net flux from A into the system, and $\mathcal{J}_B(t)$ is the net flux from PYR into B. We are interested in the difference between $\Delta(\omega)$ at finite frequencies and in the static limit ($\omega = 0$). Inasmuch as we think of efficiency in terms of reduced dissipation, we use the number

$$\epsilon(\omega) = \frac{\Delta(0) - \Delta(\omega)}{\Delta(0)} \qquad (4)$$

as a measure of the efficiency increase due to oscillations. The frequency dependence of $\epsilon(\omega)$ is characteristically different in the two cases mentioned above. When the phase difference φ is chosen so as to minimize the dissipation, at each frequency, the results are as shown in Fig. 2. For the purely dissipative case (lower curve) the number $\epsilon(\omega)$ is everywhere negative, decreasing with increasing frequency. This means that oscillatory driving always causes more dissipation than a stationary operation. However, when the system shows oscillatory relaxation, the efficiency is enhanced near resonance between the intrinsic and the driving frequency, as shown by the upper curve in Fig. 2. Note, however, that this enhancement depends crucially on properly adjusting the phase relation between A and B. Instead of minimizing the dissipation, we could also maximize it and thereby get increased dissipation at resonance. This shows that a possible role of oscillatory reactions is the sensitive regulation of dissipation.

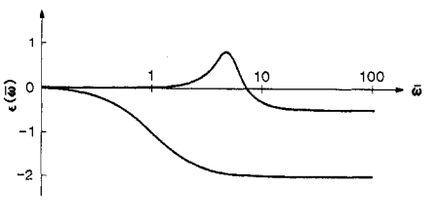

Fig. 2. Comparison of a purely dissipative system (lower curve) with one that possesses irreversible elasticity (upper curve). The efficiency $\epsilon(\tilde{\omega})$ (Eq. 4) is plotted against frequency $\tilde{\omega}$ in arbitrary units. The upper curve shows resonance behavior near $\tilde{\omega} = 5$. $\tilde{\omega}$ is a conveniently reduced frequency [for further details, see (*18*)].

These observations do not depend on linearization of the kinetic equations, nor do they require that the system's intrinsic oscillations are damped. Numerical studies with nonlinear models (*19, 20*) have shown that the resonance enhancement of efficiency is a generic feature, given proper phase adjustment, and is even more strongly pronounced in case of undamped spontaneous oscillations. In the latter case, however, large driving amplitudes tend to excite the system too strongly, so that close to resonance the efficiency may indeed be reduced. Since the glycolytic oscillations as generated in the PFK reaction are high in amplitude, it seems more advantageous for the driven oscillator at the lower end to be of the passive type rather than active in its own right.

Using specific examples we have estimated (*18*) that the relative reduction in dissipation due to this resonance effect is of the order of 10 percent. Glycolysis being a vital process, such an increase in efficiency may have warranted an evolutionary effort to develop the chemical machinery necessary for (i) generation of oscillations, (ii) phase regulation, and (iii) resonance response in the driven oscillator.

The following experimental tests are suggested in regard to the ideas presented. First, more careful concentration measurements of the various intermediates are necessary to establish firmly the free energy profile of Fig. 1. Second, the response behavior of the PK reaction, under appropriate nonequilibrium conditions, should be checked for the postulated oscillatory relaxation. Third, it is crucial to find out whether there is resonance of the PK relaxation with the PFK-generated oscillations.

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Free and Forced Diving in Birds

Abstract. *Heart rates were measured during free and forced diving on each of two species of aquatic birds: the double-crested cormorant* (Phalacrocorax auritus), *a true diver, and the Canada goose* (Branta canadensis), *a bottom feeder in shallow water. When they immersed voluntarily they showed no bradycardia, but when the same birds were forcibly held under water there was a rapid drop in heart rate to well below that at rest. This decrease indicates that there may be a large component of emotional stress in the heart rate records from previous diving studies where restrained animals were forcibly submerged.*

Experimentally submerged diving birds and mammals have usually shown an abrupt decrease in heart rate (*1*). The reduced circulation indicated by this bradycardia is generally interpreted as an oxygen saving response that allows the animal to extend its diving time. Recent observations, however, on unrestrained animals have shown either a variable degree of this bradycardia or none at all (*2–4*), and intense bradycardia unrelated to diving has been seen in a variety of mammals and birds (*5,6*). We have sought to clarify this relation by looking at the heart rate response to both free and forced diving in the same animal. We used two each of two species of trained and hand-reared seabirds, the double-crested cormorant (*Phalacrocorax auritus*) and the Canada goose (*Branta canadensis*).

The cormorants were taken as 2-week-old nestlings. The geese were hatched from the eggs of wild birds. They were hand-fed in a pen adjacent to our house and were generally treated as pets. After they reached 3 to 4 weeks of age they accompanied us on walks and swims, and even sought our company by invading the house through open doors and windows. This lack of fear they showed toward us allowed us to place instruments on them and work with them at Great Harbor in Woods Hole.

Both species learned to fly at 7 to 9 weeks of age. The cormorants also began diving spontaneously at the same time, and within a day or two were seen to catch fish and eels. But they were always receptive when we offered a fish, making it easy for us to catch them for heart rate observations during forced submersion.

We used frequency modulation radiotelemetry of the electrocardiogram (EKG) from the geese, and also from the cormorants when they were not underwater. The transmitters were thumb-sized cylinders held on the back of the bird by a wire harness and straps. The transmitter's weight was never more than 5 percent of that of the bird. The EKG leads were inserted under the breastbone close to the heart. The wire was sutured at the point of entry and also glued to the feathers.

Since radio signals do not penetrate seawater we used acoustic telemetry from the cormorants while they were on and under the surface. The geese only put their heads and necks under, so that the backpack radio remained in the air. The range of both types of transmitters was a few hundred meters (*7, 8*). The received EKG signals were heard as pitch changes of an audio tone, which was magnetically recorded for later demodulation to the heart rate curves shown (Fig. 1).

The forced dives were not prolonged, and there is little doubt that the birds would have survived for at least a minute. However, the times we used are sufficient to demonstrate the biological unnaturalness of forcing a bird underwater to study the biological significance of the heart rate response to diving.

We were able to observe the heart rates of these tame birds during, for example, feeding, running, flying, swimming, and diving. Both species easily adapted to living in the vicinity of our house and wore transmitters with no observable change in behavior after a few hours. Although both species struggled somewhat during the forced submersion, they returned quickly to voluntary diving when released. This allowed us to make the following heart rate observations.

1) Both cormorants and geese have a steady heart rate of 100 to 120 beats per minute when they are inactive. This basal rate is maintained for many hours at night when they perch quietly in the dark.

2) Increases in heart rate to twice or more of the basal rate are common when there is a disturbance. Such periods of tachycardia suggest that both species respond emotionally to people, sounds, lights, and other birds.

3) Free-ranging geese had heart rates of 140 to 160 beats per minute while swimming slowly. In moderately active cormorants that were swimming slowly, or standing on the dock while drying by gently flapping their wings, the rate was 170 to 230 beats per minute. These rates, significantly above basal, are the normal heart rates to which voluntary diving or underwater feeding rates should be compared.

4) Both of the geese eagerly ate corn (maize) placed at the bottom in shallow water. They repeatedly submerged their heads for 8- to 14-second periods. These were separated by 3 to 5 seconds in air. The heart rate while the head was under the water was the normal active one of 150 beats per minute. As the head started to come up to the surface, the rate rose rapidly and stayed at 250 to 300 until they submerged again. This onset of tachycardia precedes the actual breath (or breaths).

5) In the middle of such a series of voluntary dives we seized a goose and forcibly held it under water. The heart rate dropped immediately to the base

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