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*A CHEMICAL MECHANISM FOR OSCILLATION OF GLYCOLYTIC INTERMEDIATES IN YEAST CELLS**

BY JOSEPH HIGGINS

JOHNSON RESEARCH FOUNDATION, UNIVERSITY OF PENNSYLVANIA, PHILADELPHIA

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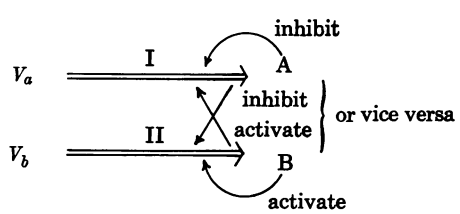
Other papers[1, 2] have described the methods for the measurements of reduced pyridine nucleotide in yeast cell suspensions, the observation of cyclic and oscillatory responses of reduced pyridine nucleotide under appropriate experimental conditions and the metabolite assay, which indicate by the crossover theorem[3] that the control site involved is phosphofructokinase. In this paper, a general chemical mechanism for producing oscillatory kinetics is briefly described (the details and proofs are given elsewhere[4]). A special case of this general mechanism, involving only the known chemistry of phosphofructokinase[5] and the neighboring metabolic reactions, provides one satisfactory explanation for the observed oscillations of the glycolytic intermediates.

General Oscillatory Mechanism.—In order for a chemical mechanism to exhibit oscillatory behavior, it is necessary that certain general types of reaction pathways exist. Let A be some chemical whose *net* rate of production (v_a) is determined by some arbitrary set of pathways labeled I,—similarly for chemical B.



Then oscillations can exist if all the following conditions apply:

(1) One of the chemicals (say B) must activate its own production (assuming

that A remains fixed). That is, increasing the concentration of B will *tend* to increase the *net* rate of production of B.

(2) The other chemical (A) must tend to inactivate its own *net* production. (This is normally true of most chemical reactions since increasing concentration increases the rate of removal of that chemical.)

(3) In addition, there must be cross coupling of opposite character. If increasing B tends to activate the *net* production of A, then increasing A must tend to inhibit the *net* production of B, or vice versa.

These conditions are illustrated in the diagram. Several points should be particularly noted. First, the conditions (1), (2), and (3) apply to the *net* rate of production; thus, many different specific mechanisms can satisfy the requirements. If the generalized mechanism is presented in more detail as

$$\begin{array}{l} \overset{v_1}{\rightarrow} \mathrm{A} \overset{v_2}{\rightarrow} \\ \\ \overset{v_3}{\rightarrow} \mathrm{B} \overset{v_4}{\rightarrow} \end{array} \qquad \text{(I)}$$

where v_1 and v_3 are specific rates of production, while v_2 and v_4 are specific rates of removal, then the net rate of production for A is given as

$$v_a = v_1 - v_2.$$

By inhibiting the specific rate of production, v_1, or by activating the removal rate, v_2, the net rate of production (v_a) will be inhibited,—similarly for $v_b = v_3 - v_4$. It is in this manner that the conditions on the net rates must be interpreted.

Second, it should be noted that these general conditions relating to the reaction pathways are only necessary conditions for oscillatory behavior; just because a mechanism satisfies these conditions does not guarantee that oscillations will occur. A full publication will treat additional conditions relating to the strength of the cross coupling in comparison to the self-coupling.[4]

Finally, it should be emphasized that the reaction pathways referred to can be of a general nature; there may be common loops or branches and large numbers of chemicals involved. So long as the requisite conditions on the self and cross coupling are satisfied, the potential oscillatory character will remain.

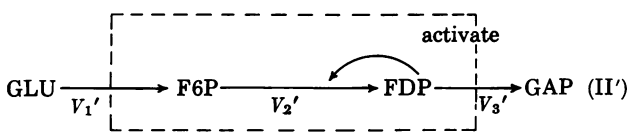
Mechanism for Glycolytic Oscillations.—A specialization of the general mechanism to describe the oscillatory behavior observed in the glycolytic system is given by equations 1–6 (Mechanism II). This mechanism has been chosen as a working hypothesis to describe the known chemistry of phosphofructokinase and the associated glycolytic intermediates.

$$\begin{array}{lll} (1) & \mathrm{GLU} & \rightharpoonup \mathrm{F6P} \\ (2) & \mathrm{F6P} + \mathrm{E_1}^* & \rightharpoonup \mathrm{E_1}^* \cdot \mathrm{F6P} \\ (3) & \mathrm{E_1}^* \cdot \mathrm{F6P} & \rightharpoonup \mathrm{E_1}^* + \mathrm{FDP} \\ (4) & \mathrm{FDP} + \mathrm{E}^+{}_1 & \rightleftharpoons \mathrm{E_1}^* \\ (5) & \mathrm{FDP} + \mathrm{E_2} & \rightharpoonup \mathrm{E_2} \cdot \mathrm{FDP} \\ (6) & \mathrm{E_2} \cdot \mathrm{FDP} & \rightharpoonup \mathrm{E_2} + \mathrm{GAP} \end{array} \qquad \text{(II)}$$

Equation 1 represents a one-step transformation of glucose to fructose-6-phosphate by a first-order reaction (the enzymatic dependence of this step has been suppressed but makes no significant difference in the results). In the second step, E_1^* represents an activated form of phosphofructokinase which combines with a substrate (F6P) to make an enzyme substrate compound. This enzyme substrate compound breaks down in the first-order reaction (3) to form the free activated enzyme and the product, FDP. FDP combines with a second enzyme E_2, representing a combination of aldolase and triose phosphate isomerase. The second-order reaction (5) forms an enzyme substrate compound $E_2 \cdot FDP$. This intermediate breaks down to form free E_2 and the product, glyceraldehyde phosphate (GAP). DPNH is omitted in this representation. Thus far, the enzymatic system contains no step which would cause oscillation. In fact, it is simply a linear sequence involving two enzyme intermediates.

The critical feature is given by equation 4, in which FDP reacts with an inactive form (E_1^+) of the enzyme to produce the active form (E_1^*). While the conversion from the active to the inactive form is treated here by making reaction 4 reversible, it is suggested that it may depend on some other intermediates, such as ADP.

A concise representation of the mechanism (II) can be written as:



where the steps contained in the dashed box are to be correlated with the general mechanism (I) such that F6P and A are associated, as are FDP and B. This specialization is identical with the mechanism (I) if we take the off-rate for (A) (i.e., v_2) to be the on-rate for (B) (i.e., v_3), that is, one and the same reaction step (namely, v_2'). The ability of the FDP to activate this step produces not only an increase in the *net* rate of FDP production, but a decrease (inhibition) in the net production of F6P. Thus, the reaction pathways satisfy the oscillatory coupling conditions.

Properties of the Reaction Mechanism.—For any mechanism, a necessary condition for sustained oscillations is that the system be in a stationary state; in this case, the glucose must be maintained at a fixed value (through some external source). For the mechanism given above, both theoretical analysis and analogue computer studies have shown that the stationary-state condition is not sufficient. Instead, there are limited ranges of rate constants and glucose concentrations for which sustained oscillations will occur. Outside of these ranges, the system simply proceeds to a nonoscillatory state. Typical computer solutions for the sustained oscillations are shown in Figure 1 together with the associated phase plane plots. (The computer studies and solutions[6, 7] presented here were obtained on the Johnson Research Foundation electronic analogue computer, Mark II, with the aid of Mr. Ranganzes.) If, under conditions such that sustained oscillations would occur in the stationary state, the glucose concentration is allowed to decay, then the kinetics will, in general, display damped oscillations as the system proceeds to the equilibrium state. Computer solutions demonstrating this are shown in Figure 2, where it may be noted that the phase plane trajectories become spirals.

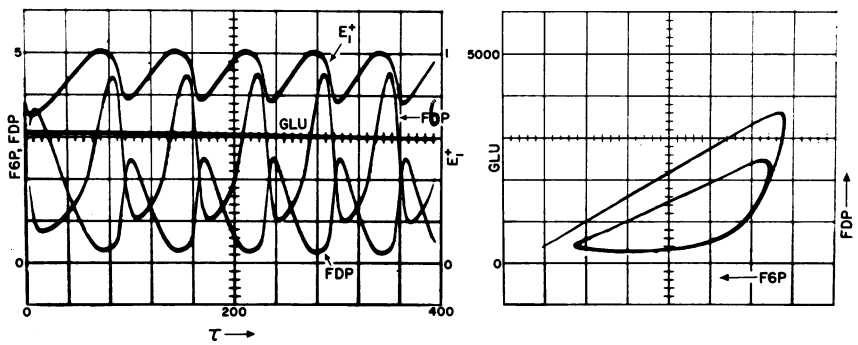


FIG. 1.—Sustained oscillations under condition of virtually constant glucose concentration. The right-hand figure shows the associated phase plane trajectory. The concentration scales are indicated in normalized units; both enzymes have a scale of 1.0. The rate constants, in normalized units, are $k_1 = 0.8 \times 10^{-5}$, $k_2 = 0.45$, $k_3 = 0.6$, $k_4 = 0.6 \times 10^{-1}$, $k_{-4} = 0.5$, $k_5 = 0.16$, $k_6 = 0.4 \times 10^{-1}$. The time scale is in normalized units, and the initial conditions are: GLU (as shown), F6P = 5, $E_1^* = 1$, $E_2 = 1$, all others zero. (Normalized units are connected to real units as follows: if α is the unit of time and β the unit of concentration, then first-order rate constants are divided by α, and second-order rate constants are divided by $\alpha\beta$.)

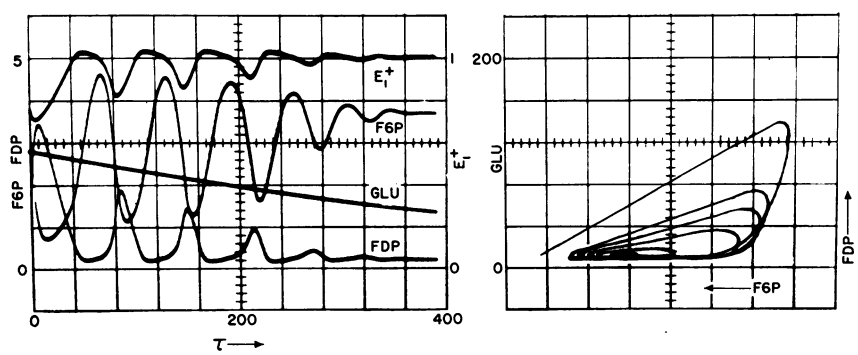


FIG. 2.—Damped oscillations arising through depletion of glucose. All conditions the same as Fig. 1 except the glucose scale (as indicated) and $k_1 = 0.2 \times 10^{-3}$.

The kinetic curves do not need to demonstrate oscillations at the beginning of the reaction. Rather, the oscillations occur when the glucose in the course of its utilization passes through the critical concentration ranges. Since the frequency of oscillations is virtually independent of glucose concentration, the number of oscillations observed depends on the rate at which the glucose passes through the critical range, as illustrated in Figure 3.

A similar situation prevails when the rate constants are considered to be pseudo first-order and are dependent on the concentrations of other glycolytic intermediates (such as ATP and ADP). Then the possibility of oscillations will depend on whether or not the concentrations of these additional intermediates are within certain definite ranges. In addition, if the concentrations of these intermediates can change during the course of the reaction, potentially sustained oscillations may again become damped oscillations.

Chemicals connected to the basic oscillatory couple (in this case, F6P-FDP) by appropriate reaction pathways will also exhibit oscillatory kinetics. If GAP is considered to be an intermediate in a sequence of reactions (as it actually is), then it will oscillate; similarly for intermediates between GLU and F6P which are

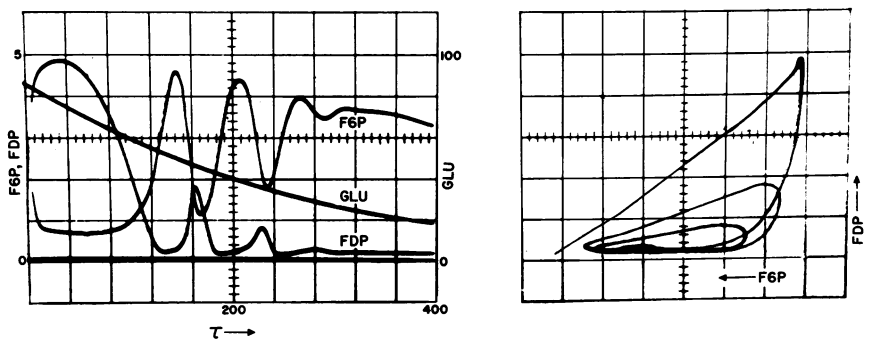


FIG. 3.—Kinetic response showing passage of glucose through critical range for oscillations. All conditions are the same as Fig. 1 except the glucose scale (as indicated) and $k_1 = 0.4 \times 10^{-3}$.

effected by F6P concentrations as through reversible reactions or product-sensitive enzymes.

Relation to Experimental Data.—The proposed mechanism is entirely consistent with the known chemistry and the qualitative kinetic behavior observed experimentally. In addition, it offers a satisfactory explanation of many other aspects relating to the experimental conditions required for obtaining the oscillatory response. That such response is thus far obtained only under special conditions of cell growth, as in log phase cells, and anoxia or cyanide are required to induce the oscillations, is interpreted as placing the concentrations of the control intermediates (ATP, DPNH, etc.) within the requisite ranges. The experimental dependence of the number of oscillations on the glucose concentration can be explained similarly. As previously indicated, the oscillations observed in the other glycolytic intermediates can be understood in terms of the known reaction pathways connecting them to the F6P-FDP oscillatory couple.

Discussion.—Previous attempts to explain periodic reactions have been limited to one or two special types of mechanisms, such as that of Lotka[9, 10] or, more recently, Spangler and Snell.[11] In contrast, the generalized mechanism now offers a plethora of mechanisms which can exhibit sustained oscillations. This result is of particular importance for cellular reactions, as in the case of glycolysis, where there exists a number of chemical feed-back pathways which could act as potential oscillators under the proper conditions.

It must be kept in mind that only damped oscillations have thus far been observed experimentally, and the number of possible mechanisms which exhibit such response is even greater. As already indicated, potentially sustained oscillatory mechanisms will exhibit damped oscillations when the stationary-state conditions are not maintained. For the particular experiments performed in these studies, which deal with cell populations, there is the additional possibility that damped oscillations appear to arise through a loss of synchrony between sustained oscillations associated with individual cells. In such a case, damped oscillation would occur at times of artificially induced synchrony as caused by anoxia. However, observations with the microfluorometric technique[8] (experiments kindly carried out by Dr. David Epel) indicate that single cells are not continuously oscillating.

In addition to these considerations, there are a large number of mechanisms which *cannot* exhibit sustained oscillations under stationary conditions, but which do

show damped oscillations in the approach to the stationary state as a part of their kinetic response. For certain ranges of the rate constants, the proposed mechanism (II) will have this property.

Since most of the glycolytic intermediates exhibit oscillatory behavior, the choice of F6P and FDP as the basic oscillatory couple is certainly open to question. Theoretically, it appears to be one of the simplest hypotheses consistent with the known chemistry. Also, application of the crossover theorem to the experimental data has indicated that phosphofructokinase is the control site. It is expected that further theoretical and computer studies, currently in progress, will provide other tests for detecting the basic couple. The achievement of the oscillatory response *in vitro* appears particularly important.[12] It would offer a direct experimental approach for clarifying the reaction mechanism and the role of the control intermediates.

Conclusions and Summary.—A generalized chemical mechanism is given which provides a basis for the derivation of specific reaction mechanisms which can exhibit oscillatory behavior. The general mechanism was studied in relation to the pathways of the glycolytic system, and led to a specific reaction mechanism, consistent with the known chemistry and offering a satisfactory explanation of the observed oscillatory kinetics. The specific mechanism consists of the feed-in of a substrate through a first-order (or enzymatic reaction), a product-activated enzyme step, and an enzymatic step for removing this product. Under the stationary-state conditions, the oscillatory behavior can be sustained. It appears that all previously suggested, purely chemical oscillatory mechanisms have invoked reaction steps with stoichiometry greater than one, thus considerably restricting their applicability to experimental phenomena. We believe the specific mechanism (II) given here is the first example to require only normal stoichiometry. Its direct application to a large number of known chemical reactions is apparent.

The author wishes to point out that this research was stimulated by Dr. Britton Chance and that many of the comments presented here are directly attributable to him. His contribution to the formulation of the specific mechanism (II) in terms of the known chemistry of the glycolytic system was most important.

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