Proc. Nat. Acad. Sci. USA
Vol. 72, No. 10, pp. 3829–3833, October 1975
Biochemistry

Control of oscillating glycolysis of yeast by stochastic, periodic, and steady source of substrate: A model and experimental study

(phosphofructokinase/entrainment/allosteric model/dissipative structures)

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Communicated by Manfred Eigen, April 28, 1975

ABSTRACT **Type and range of entrainment of glycolytic oscillations by a periodic source of substrate are determined experimentally in yeast extracts. Subharmonic entrainment proves the nonlinear nature of the glycolytic oscillator. Random variation of the substrate input yields sustained oscillations of irregular waveform and stable period. The results agree with the predictions of an allosteric model for phosphofructokinase (EC 2.7.1.11; ATP:D-fructose-6-phosphate 1-phosphotransferase), which is the enzyme responsible for periodic operation of glycolysis. A comparison between model and experiment in the case of a constant source of substrate further indicates that the oscillatory dynamics of the glycolytic system can satisfactorily be described by the phosphofructokinase model.**

After the discovery of glycolytic oscillations in yeast and later in extracts of heart, mechanistic studies (for summary see ref. 1) led to the identification of phosphofructokinase (PFK) (EC 2.7.1.11; ATP:D-fructose-6-phosphate 1-phosphotransferase) as the enzyme periodically generating its products, ADP and fructose-1,6-bisphosphate (Fru-1,6-P_2). Furthermore, the mechanism of propagation of the periodic change of activity of PFK along the enzymic reaction sequence of glycolysis through the adenylate system was elucidated (2, 3). The allosteric properties of PFK and the positive feedback exerted on it by a reaction product were described and suggested as being responsible for its periodic operation.

A companion study of a model for an allosteric enzyme activated by the product showed indeed (4, 5) that this system undergoes sustained oscillations corresponding to a limit cycle around a nonequilibrium, unstable, stationary state (6). The analysis of the model yields insight into the molecular mechanism of instability and into the dependence of oscillations on flux and enzyme parameters (5). Thus, experimental and theoretical results demonstrate that the cooperative and regulatory properties of PFK give rise to a temporal dissipative structure (6).

Whereas, earlier studies have shown that in an extract of yeast (7, 8), as well as in yeast cells (9), an oscillatory domain can be identified on application of a variety of steady rates of substrate input, the question arises, whether the oscillating system can be entrained by a periodic source of substrate and how it reacts to random perturbation. This paper reports a model analysis describing the effect of stochastic input of substrate on limit cycle behavior and conditions for entrainment of the oscillating system, together with the experimental verification of the theoretical predictions. Further agreement of the oscillatory behavior of the model with experiments performed with a constant source of substrate is presented. The results are compared to findings on circadian rhythms.

Abbreviations: PFK, phosphofructokinase (EC 2.7.1.11); Fru-6-*P*, fructose-6-phosphate; Fru-1,6-P_2, fructose-1,6-bisphosphate.

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METHODS

Yeast [*Saccharomyces carlsbergensis* (ATCC 4228)] was grown under anaerobic conditions and extracted as described elsewhere (10). The extracts were pooled, with an average protein content of 50 mg/ml. The protein concentration was determined by the procedure described in ref. 11. The experimental volume was 2 ml. NADH fluorescence (in arbitrary units) and absorbance (in extinction units; sample path length, d = 1 cm) were recorded at 30°. An injection technique was used for continuous addition of glucose to the yeast extracts (10). Stochastic and periodic variation of the rate of substrate injection were simulated by stepwise changes of input controlled by a mechanical gear system. Stochastic variation of the rate of input was obtained according to a random number table (12). In model simulations, Eq. 1 were integrated on an IBM 370-165 computer by means of the Continuous System Modeling Program for different values of the source term σ_1. Stochastic variation of this parameter was obtained using a random number generator yielding white noise.

RESULTS

Theory

In the frame of the concerted transition theory (13) an allosteric model for the oscillatory PFK reaction has been developed (4, 5). The model is that of an open K-V system in which the product is a positive effector of the dimer enzyme§ (for details see refs. 5 and 14). In the homogeneous case, i.e., when the system is continuously stirred as in experiments with yeast extracts, diffusion can be neglected, and the time-evolution of the metabolite concentrations is described by the following equations (5, 14):

$$d\alpha/dt = \sigma_1 - \sigma_M\Phi \qquad d\gamma/dt = \sigma_M\Phi - k_s\gamma \quad [1]$$

where

$$\Phi = \frac{\alpha e(1 + \alpha e)(1 + \gamma)^2 + L\Theta\alpha ce'(1 + \alpha ce')}{L(1 + \alpha ce')^2 + (1 + \gamma)^2(1 + \alpha e)^2} \quad [2]$$

with $e = 1/(\epsilon + 1)$ and $e' = 1/(\epsilon' + 1)$. Furthermore, α and

§ Extension of the model to the case of n protomers and two substrates leads to similar conclusions for periodic behavior (15).

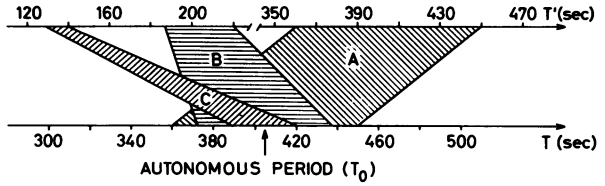

FIG. 1. Domains of entrainment of the enzyme model by the fundamental frequency (A), ½-harmonic (B), and ⅓-harmonic (C) of a sinusoidal source of substrate $\sigma_1 = [0.5 + 0.25 \sin (2 \pi t/T)]$ sec^{-1}. In the given range, no entrainment takes place outside these domains, which extend symmetrically around $T_0 = 406$ sec; T denotes the period after entrainment. The diagram is calculated with the following numerical values of model parameters (8, 21): $L = 10^7$ (allosteric constant), $c = K_R/K_T = 10^{-5}$ (nonexclusive binding coefficient of the substrate), $\epsilon = k/d = 10^{-3}$ (normalized catalytic constant of R state), $\epsilon' = k'/d' = 10^{-3}$ (normalized catalytic constant of T state), $\Theta = k'/k = 1$ (ratio of catalytic constants of T and R states), $\sigma_M = (2kD_0)/K_R = 8$ sec^{-1} (normalized maximum enzyme activity), $k_s = 0.1$ sec^{-1} (rate constant for product sink), $K_R = d/a = 5 \times 10^{-5}$ M (dissociation constant of enzyme substrate and enzyme product complexes in R state). Similar results are obtained for $\epsilon = \epsilon' = 0.1$. For remaining parameters, the mean values chosen for simulations are: $K_T = d'/a' = 5 \times 10^{-3}$ M (dissociation constant of enzyme substrate complexes in T state), $a = 10^8$ M^{-1} sec^{-1} (association rate constant for R state), $d = 5000$ sec^{-1} (dissociation rate constant for R state), $k \leq 500$ sec^{-1} (catalytic constant of R state), and $D_0 = 5 \times 10^{-7}$ M (total enzyme concentration).

γ denote, respectively, the concentrations of substrate (ATP or Fru-6-P) and product (ADP or Fru-1,6-P_2) of the enzyme reaction normalized by division through the dissociation constant K_{Rj}, σ_1 denotes the normalized injection rate of substrate, and k_s the rate constant for the sink of the product (for further parameters, see legend of Fig. 1; also see refs. 5 and 14).

Under conditions of constant input, the system described by Eq. 1 undergoes limit cycle oscillations in a finite domain of σ_1 values (5). In a case for which a close agreement is reached with the experiments in yeast extract (see Table 2 below), this domain of sustained oscillations extends from $\sigma_1 = 1.05\ k_s$ to $13\ k_s$.

We first consider here a source rate fluctuating in the range 5–8 k_s, in the middle of the oscillatory domain. For $L = 5 \times 10^6$, $c = 10^{-2}$, $\epsilon = 0.1$, $\epsilon' = \Theta = 0$, $\sigma_M = 10^2$ sec^{-1}, and $k_s = 0.1$ sec^{-1}, the corresponding range of periods for constant input extends from 213.6 sec to 154.1 sec. When σ_1 varies stochastically, the period of the oscillations ranges from 182 to 172 sec, with a mean period of 177.5 sec. Thus, the allosteric enzyme retains periodic behavior and acts, furthermore, as a narrow filter centered at the mean frequency. Oscillations persist also with input values that extend partly over the range corresponding to a stable steady state. In contrast to the stability of the period, noise renders the amplitude irregular.

The response of the system towards a periodic source of substrate depends on the relative magnitude of the period T' of the input with respect to the autonomous[¶] period T_0 of the enzyme. In order to simulate the experiments closely, we consider a set of parameter values yielding an autonomous period of 406 sec for $\sigma_1 = 5\ k_s = 0.5$ sec^{-1}, and investigate the effect of a periodic source by means of a sinusoidal expression for σ_1. The results of simulations for T' varying in the range 120–600 sec are summarized in Fig. 1, where T denotes the period of the driven system. The enzyme entrains to the driving frequency ($T = T'$) in the range $360 \leq T' \leq 450$ (in sec); this range widens to $345 \leq T' \leq 475$ (in sec) when the amplitude of the sinusoidal source increases from 0.25 to 0.35. Subharmonic entrainment (16) by the ½-harmonic ($T = 2T'$) and by the ⅓-harmonic ($T = 3T'$) of the external frequency occurs for $187 \leq T' \leq 220$ (in sec) and for $130 \leq T' \leq 140$ (in sec), respectively. Harmonics of the external frequency fail to entrain the system. For longer periods T', modulation of the enzyme oscillations by the source is observed, and the system displays double periodicity (Fig. 7b): one period is of the order of T_0, whereas the second is equal to T'.

[¶] The autonomous period T_0 of the enzymatic oscillator is defined as the period of the oscillations obtained with a constant source rate equal to the temporal mean value of the periodic or stochastic rate of substrate input.

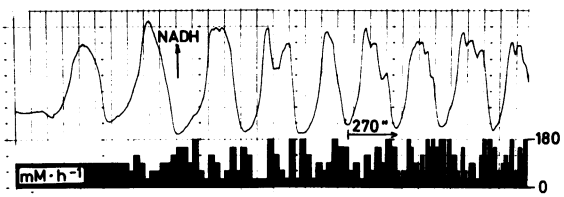

FIG. 2. NADH fluorescence (upper trace) in a yeast extract supplied with a stochastic input of substrate (lower trace). The injection rate varies between 30 and 180 mM × hr^{-1} of glucose. The glycolytic oscillator operates with a stable period of about 270 sec, depending only on the average rate of substrate injection.

In the absence of entrainment, period and amplitude of the oscillations are irregular, whereas their phase relation with the periodic input varies as a function of time. In contrast, the three factors are constant in case of entrainment. Moreover, the phase difference between the entrained oscillatory enzyme and the source is fixed, regardless of initial conditions, but varies with the driving frequency. The amplitude of the oscillations increases by about 10% upon entrainment.

Keeping σ_1 constant and assuming a sinusoidal expression for parameter σ_M, which includes the total enzyme concentration (see legend of Fig. 1), allows us to test the effect of periodic enzyme synthesis on limit cycle behavior. The period of epigenetic oscillations is usually larger by one order of magnitude than the period of metabolic oscillations (1). In this case, a modulation of the periodicities quite similar to that shown in Fig. 7b is obtained.

Experiments

Stochastic variation of the rate of substrate input, after a short steady rate of injection, leads to sustained periodic behavior with small variation of the amplitude and some irregularities in the waveform (Fig. 2). As predicted by the

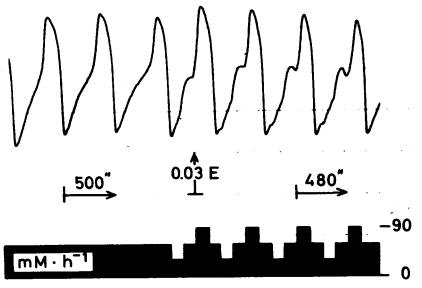

FIG. 3. Record of NADH absorbance (upper trace) in a yeast extract entrained by a periodic glucose injection rate (lower trace). $T_0 = 320$ sec, $T' = 280$ sec. Resulting period: $T = T' = 280$ sec.

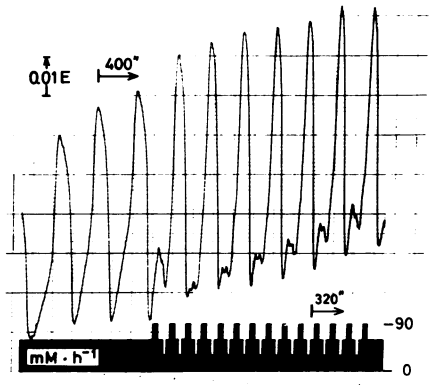

FIG. 4. NADH absorbance (upper trace) in a yeast extract entrained by the ½-harmonic of a periodic glucose injection rate (lower trace). T_0 = 400 sec, T' = 160 sec. Resulting period: T = 2 T' = 320 sec.

model, the oscillation is restricted to a narrow range around the autonomous period.

The result of periodic variation of the rate of substrate injection is shown in Figs. 3–5 for three different input periods. In each experiment the system was first induced to oscillate by a constant rate of substrate injection, then the periodic variation was started. The experiments demonstrate an instantaneous frequency response of glycolytic oscillations. Entrainment by the fundamental frequency (Fig. 3), by the ½-harmonic (Fig. 4), and by the ⅓-harmonic (Fig. 5) is observed. A situation corresponding to the absence of entrainment is given in Fig. 6. The experiment was started by a continuous rate of input resulting in oscillations, being followed by a rate variation to a nonentraining frequency. Here, period, amplitude, and phase difference between source rate and oscillating NADH level are irregular, in contrast to the situation of Figs. 3–5. When the period of the rate of input is sufficiently longer than the autonomous period, both periodicities are displayed separately by the system (Fig. 7a), quite similarly to the behavior of the model (Fig. 7b). The results of a series of experiments analogous to those demonstrated by the figures are summarized in Table 1, where the type of interaction between autonomous and source oscillations is shown to depend on the ratio of their periods.

DISCUSSION

The data obtained from the simple allosteric model for PFK agree well with the experiments performed in yeast extract. Under stochastic input conditions (more likely representing true conditions *in vivo* than the constant source) model and experiment show the effect of a narrow band-pass filter centered at the mean autonomous frequency, keeping the period stable in spite of short time variations of the source rate.

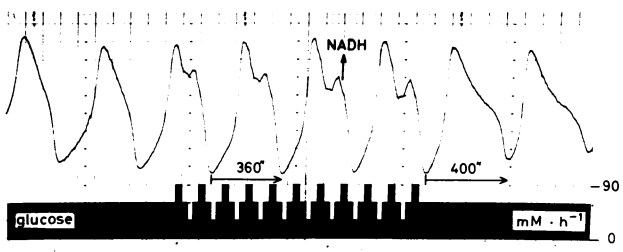

FIG. 5. NADH fluorescence (upper trace) in a yeast extract entrained by the ⅓-harmonic of a periodic input of glucose (lower trace). T_0 = 400 sec, T' = 120 sec. Resulting period: T = 3 T' = 360 sec.

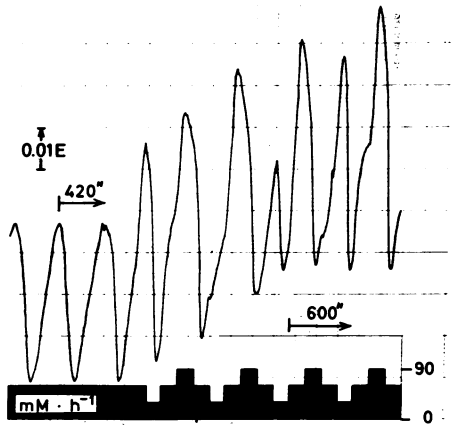

FIG. 6. NADH absorbance (upper trace) in a yeast extract that fails to be entrained by a periodic rate of glucose injection (lower trace). T_0 = 420 sec, T' = 600 sec. Resulting period: T not constant.

This property could be of physiological significance in providing reliable timing mechanisms at the cellular level.

For a periodic input of substrate, the domains of entrainment $0.89 \leq T'/T_0 \leq 1.11$ and $0.85 \leq T'/T_0 \leq 1.17$ computed theoretically for a sinusoidal source amplitude of 0.25 and 0.35, respectively, compare with the slightly larger range observed in the experiments for entrainment by the fundamental frequency (Table 1). The agreement extends to the case of subharmonic entrainment and to the modulation of autonomous oscillations by a source of longer period.

The results of simulations and experiments with a constant

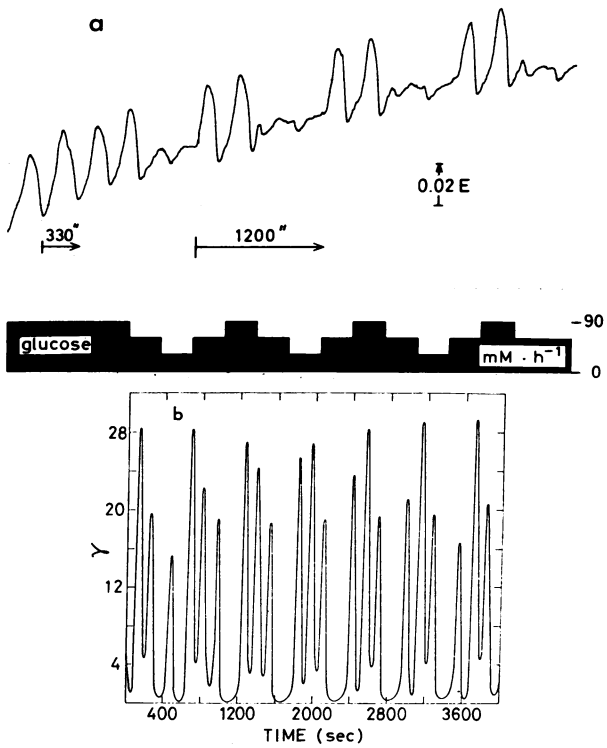

FIG. 7 (a) NADH absorbance (upper trace) in a yeast extract modulated by a periodic rate of glucose injection (lower trace). T_0 = 330 sec, T' = 1200 sec. Clear separation of autonomous and external periods. (b) Modulation of the model periodicities (T_0 = 183 sec) by a sinusoidal source of substrate of longer period (T' = 600 sec). The curve represents the periodic variation of concentration γ for $L = 10^6$, $c = 10^{-5}$, $\epsilon = \epsilon' = 10^{-3}$, $\Theta = 1$, $\sigma_M = 4$ sec^{-1}, and k_s = 0.1 sec^{-1}. Initial conditions: $\alpha = 40$, $\gamma = 8$. The sinusoidal expression for the input is $\sigma_1 = [0.7 + 0.5 \sin (2\pi t/600)]$ sec^{-1}.

Table 1. Interaction of the glycolytic oscillator with a periodic source of substrate

Relation between T' and T_0	Interaction
$T'/T_0 \approx 1/n$ (n = 2,3...)	Entrainment by the $1/n$ subharmonic of the input frequency (Figs. 4 and 5)
$0.7 \leq T'/T_0 \leq 1.2$	Entrainment by the fundamental frequency of the input (Fig. 3)
$1.2 < T'/T_0 < 1.6$	No entrainment (Fig. 6)
$T'/T_0 > 3$	Double periodicity: separation of autonomous and input frequencies (Fig. 7a)

input are summarized in Table 2. There is no serious discrepancy between model and experiment for the oscillatory range of substrate input, for period and amplitude as well as for the periodic change in PFK activity. Furthermore, the phase-shift experiments give identical results and demonstrate the sensitivity of the enzymic source of oscillation towards its controlling ligand. The accord between the oscillations exhibited by glycolysis and by the PFK model leads to the conclusion that the dynamic behavior of a complex system can be reduced to the molecular properties of a single protein species operating as a master enzyme in a biochemical pathway.

The conditions necessary for the occurrence of sustained oscillations in open systems (3, 6, 18, 19) are satisfied in glycolysis. The far from equilibrium operation of the PFK reaction in yeast has indeed been demonstrated (8). Furthermore, enzyme cooperativity and positive feedback introduce nonlinearities in the evolution equations of metabolite concentrations. The demonstration of subharmonic entrainment offers a further proof of the nonlinear nature of the glycolytic oscillator, since this type of response to a periodic input is not observed in linear systems (16). Thus, glycolysis offers the best known example of temporal organization in a biochemical pathway beyond a nonequilibrium instability. Spatiotemporal dissipative structures in the form of chemical waves may also arise in the PFK reaction, as shown in a theoretical study (20) where the role of diffusion is considered.

Since our knowledge of the molecular properties of PFK in yeast is still limited, the enzyme model is partly based on the PFK of *Escherichia coli* (21). However, experimental data presently available on the yeast enzyme (22) indicate that the application of figures from *E. coli* to the yeast system is justified. Earlier studies (8) have shown that the yeast enzyme, underoscillatory conditions, behaves as a K system according to Monod *et al.* (13), and that no oscillation of glycolysis is observed whenever the kinetics of the enzyme are Michaelian, i.e., in the presence of saturating concentrations of Fru-6-*P*, of ammonium ions, or at high pH (10). Thus, the experimental studies clearly indicate the cooperative nature of the control mechanism underlying the periodic change of PFK activity under oscillating conditions. The role of cooperativity in the mechanism of instability is well illustrated by the model, where a necessary condition for sustained oscillations is a Hill number close to the maximum value at the unstable stationary state (5). A higher number of protomers leading to higher Hill numbers does not change the properties of the system essentially (15).

The study of the entrainability of glycolytic oscillations presents analogies with findings on circadian rhythms. Entrainment of circadian rhythms by illumination or temperature cycles has been observed in both unicellular and multicellular organisms. The range of entrainment of these rhythms to the driving frequency extends from 18 to 30 hr (23). These limits correspond to the domain $0.75 \leq T'/T_0 \leq 1.25$ comparable to that observed for glycolysis (see Table 1). Subharmonic entrainment by light-dark cycles of 12, 8, and

Table 2. Comparison of oscillatory behavior for a constant source of substrate

Sustained oscillations	Model (4, 5)	Experiment
Oscillatory range of substrate injection rate (v_i)	19–246 mM/hr[a]	20–160 mM/hr[b] (8)
Period	Of the order of min; decreases by a factor ≥ 0.1 as v_i increases	Of the order of min; decreases by a factor ≥ 0.1 as v_i increases (8)
Amplitude	In the range 10^{-5} –10^{-3} M; passes through a maximum as v_i increases	In the range 10^{-5} –10^{-3} M; passes through a maximum as v_i increases (8)
Periodic change in PFK activity (in % V_M)	Minimum: 0.95; maximum: 73; mean: 17.5; activation factor[c,d]: 77	Minimum: 1; maximum: 80; mean: 16; activation factor[d]: 80 (8)
Phase-shift by ADP	Delay[e] of 1–2 min upon addition of 0.7 mM ADP (14 units of γ) around the minimum of ADP oscillations of 5 min period; small phase advance when the addition precedes ADP maximum	Delay[e] of 1.5 min upon addition of 0.7 mM ADP at the minimum of ADP oscillations of 5 min period; small phase advance when the addition precedes ADP maximum (17)

[a] The data correspond to the range $\sigma_1 = 0.105$ sec^{-1} to 1.3 sec^{-1} obtained for $L = 5 \times 10^6$; smaller ranges are found for lower values of L (see Fig. 3.11 in ref. 5). The rate v_1 is equal to the product $\sigma_1 K_R$, with $K_R = 5 \times 10^{-5}$ M (5).

[b] The range extends beyond 200 mM/hr at 100 mM phosphate.

[c] Obtained for a K system ($\Theta = 1$) in the middle of the oscillatory domain, for $\sigma_1 = 0.5$ sec^{-1}.

[d] Larger values for the activation factor, defined as the ratio of maximum through minimum activity, are found at low values of v_1 in the oscillatory domain.

[e] The small peak of ADP (NADH) induced upon titration, is not considered as phase advance unless its amplitude approaches the unperturbed maximum of the oscillations.

6 hr periods to a 24 hr period has also been observed (23), often being referred to as entrainment by frequency demultiplication. As to the effect of stochastic disturbance of periodic behavior, circadian rhythmicity in the cell division rhythm of *Euglena*, which can be entrained by light-dark cycles, has been shown to persist in cultures exposed to a random illumination regime (24). Entrainment of the same rhythm by light-dark cycles in the domain 20–28 hr and frequency demultiplication are also observed (24). The similarity with the glycolytic response to random and periodic stimuli is of special interest in view of the conjecture (25) that metabolic oscillations of the limit cycle type are responsible for the periodicity of mitosis. It is pertinent to note in that respect that the arc discontinuity (25) characteristic of the PFK model is somewhat similar to that of the mitotic oscillator of *Physarum polycephalum* (25).

The putative link between high-frequency oscillations, such as those encountered in glycolysis, and circadian rhythms has often been emphasized (26). For given values of source and sink, the possibility of achieving circadian periods by decreasing the concentration of an oscillatory enzyme is ruled out by the PFK model (5). Coupling enzyme oscillators by diffusion also fails to elongate the period of the phenomenon in this model (20). However, the frequency can be shifted to any biologically relevant value by fitting source rate, sink, and enzyme activity appropriately.

Entrainment of metabolic oscillations offers a means of synchronizing cell populations, as demonstrated for oscillating glycolysis in yeast (17, 27). Similar patterns of entrainment can be expected in *Dictyostelium discoideum*, where oscillations controlled by cyclic AMP (28) play an essential role in the process of cellular aggregation (29), since the mechanism of these periodicities resembles that of glycolysis (30).

We thank Drs. R. Lefever, G. Nicolis, and Th. Plesser and Mr. V. Schwarzmann for valuable discussions. A.G. was supported by an EMBO fellowship during his stay in the laboratory of Prof. S. R. Caplan at the Weizmann Institute of Science, and by the Max-Planck-Society during his stay at the Max-Planck-Institut Dortmund.

1. Hess, B. & Boiteux, A. (1971) *Annu. Rev. Biochem.* **40**, 237–258.
2. Boiteux, A. & Hess, B. (1973) in *Biological and Biochemical Oscillators*, eds. Chance, B., Pye, E. K., Ghosh, A. K. & Hess, B. (Academic Press, New York and London), pp. 243–252.
3. Boiteux, A. & Hess, B. (1975) *Faraday Symp.* **9**, in press.
4. Goldbeter, A. & Lefever, R. (1972) *Biophys. J.* **12**, 1302–1315.
5. Goldbeter, A. & Nicolis, G. (1975) *Progr. Theor. Biol.* **4**, in press.
6. Glansdorff, P. & Prigogine, I. (1971) *Thermodynamic Theory of Structure, Stability and Fluctuations* (Wiley Interscience, New York).
7. Hess, B. & Boiteux, A. (1968) in *Regulatory Function of Biological Membranes*, ed. Järnefeldt, J. (Elsevier Publ. Co., Amsterdam-London-New York), pp. 148–162.
8. Hess, B., Boiteux, A. & Krüger, J. (1969) *Adv. Enzyme Regul.* **7**, 149–167.
9. von Klitzing, L. & Betz, A. (1970) *Arch. Mikrobiol.* **71**, 220–225.
10. Hess, B. & Boiteux, A. (1968) *Hoppe-Seyler's Z. Physiol. Chem.* **349**, 1567–1574.
11. Beisenherz, G., Boltze, H. J., Bücher, Th., Czok, R., Garbade, K. H., Meyer-Arendt, E. & Pfleiderer, G. (1973) *Z. Naturforsch. Teil B* **8**, 555–577.
12. Snedecor, G. W. (1956) in *Statistical Methods* (Iowa State University Press, Ames), pp. 10–14.
13. Monod, J., Wyman, J. & Changeux, J. P. (1965) *J. Mol. Biol.* **12**, 88–118.
14. Goldbeter, A. (1974) *FEBS Lett.* **43**, 327–330.
15. Plesser, Th. (1975) *Faraday Symp.* **9**, in press.
16. Hayashi, C. (1964) *Nonlinear Oscillations in Physical Systems* (McGraw-Hill, New York).
17. Pye, K. E. (1969) *Can. J. Bot.* **47**, 271–285.
18. Prigogine, I. (1967) *Introduction to Thermodynamics of Irreversible Processes* (Wiley Interscience, New York).
19. Nicolis, G. (1971) *Adv. Chem. Phys.* **19**, 209–324.
20. Goldbeter, A. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 3255–3259.
21. Blangy, D., Buc, H. & Monod, J. (1968) *J. Mol. Biol.* **31**, 13–35.
22. Tamaki, N. & Hess, B. (1975) *Hoppe-Seyler's Z. Physiol. Chem.* **356**, 399–415.
23. Bruce, V. G. (1960) *Cold Spring Harbor Symp. Quant. Biol.* **25**, 29–48.
24. Edmunds, L. N. & Funch, R. (1969) *Science* **165**, 500–503.
25. Kauffmann, S. (1974) *Bull. Math. Biol.* **36**, 171–182.
26. Winfree, A. T. (1975) *Nature* **253**, 315–319.
27. Ghosh, A. K., Chance, B. & Pye, K. E. (1971) *Arch. Biochem. Biophys.* **145**, 319–331.
28. Gerisch, G. & Hess, B. (1974) *Proc. Nat. Acad. Sci. USA* **71**, 2118–2122.
29. Gerisch, G., Malchow, D. & Hess, B. (1974) in *Biochemistry of Sensory Functions*, ed. Jänicke, L. (Springer-Verlag Berlin, Heidelberg, New York), pp. 279–298.
30. Goldbeter, A. (1975) *Nature* **253**, 540–542.