Quasiperiodicity Route to Chaos in a Biochemical System

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ABSTRACT The numerical study of a glycolytic model formed by a system of three delay differential equations reveals a quasiperiodicity route to chaos. When the delay changes in our biochemical system, we can observe the emergence of a strange attractor that replaces a previous torus. This behavior happens both under a constant input flux and when the frequency of the periodic substrate input flux changes. The results obtained under periodic input flux are in agreement with experimental observations.

INTRODUCTION

Transitions to chaos in dissipative systems differ in the way in which the signal behaves before becoming completely chaotic. As a first approximation to the true variety of transition scenarios, three classical approaches to chaos may be considered. The best known is Feigenbaum's route (1978) generated by pitchfork bifurcations, which relates the emergence of new periodic behavior by period doubling to a universal law of functional composition.

Another approach to chaos is the so-called intermittency route (Pomeau and Manneville, 1979). In this case the signal alternates randomly between a regular regime and irregular bursts. The average number of these irregular bursts may be increased through the variation of an external control parameter until the signal becomes completely chaotic. This behavior has been observed previously in our biochemical system (de la Fuente et al., 1996).

This process offers a continuous route from regular to chaotic motion (Schuster, 1984): the system passes from periodic oscillation to a chaotic system of motion without developing the Feigenbaum cascade of period-doubling bifurcations (Feigenbaum, 1978).

A third possibility is the Ruelle-Takens-Newhouse route (Ruelle and Takens, 1971; Newhouse et al., 1978). They have showed that by means of two Hopf bifurcations, regular motion may become highly unstable and be replaced by a strange attractor. For a given value of the control parameter a first Hopf bifurcation generates a limit cycle from a fixed point. A second Hopf bifurcation intruduces a new fundamental frequency (w_2) into the system. In this condition the trajectory can explore additional dimensions that correspond to a trajectory on a torus. When the quotient w_1/w_2 is rational, the trajectory closes after one cycle and is periodic. When w_1/w_2 is irrational, the motion is quasiperi-

odic, i.e., the trajectory never closes and covers the whole torus. The increment of the control parameter through another bifurcation value is followed by the destruction of the torus and the emergence of a strange attractor. The quasiperiodicity route to chaos is well verified experimentally in chemical systems (Dubois et al., 1982; Hudson et al., 1989; Scott, 1991).

Quasiperiodic behavior and chaos in the glycolytic subsystem have been observed by measuring the NADH fluorescence of glycolyzing yeast extracts under sinusoidal glucose input flux (Hess et al., 1990). Indeed, quasiperiodicity is obtained at low amplitudes of the input flux (Richter and Ross, 1980; Markus et al., 1985b), and chaos is obtained at high amplitudes of the input flux and for input frequencies between 2 and 3 times the frequency of the autonomous oscillations (Hess et al., 1984; Markus et al., 1985a).

In this paper we analyze the solutions of a glycolytic model formed by a system of three functional differential equations with delay (De la Fuente et al., 1995). Our results reveal the phenomenon of a quasiperiodicity route to chaos.

When the delay changes in our biochemical system, we can observe the emergence of a strange attractor that replaces a previous torus. This behavior happens both under a constant input flux and when the frequency of the periodic substrate input flux changes. The results obtained under periodic input flux are in agreement with experimental observations.

THE MODEL

In the present work we have used a model that represents a multienzymatic instability-generating reactive system with the enzymes arranged in series (Fig. 1). As shown in the diagram, the metabolite S is transformed by the first enzyme E_1 into the product P_1. The enzymes E_2 and E_3 are allosteric, and transform the substrates P_1 and P_2' into the products P_2 and P_3, respectively. The step $P_2 \to P_2'$ represents a particular catalytic activity, which is reflected in the dynamical system by means of the functional variable β'.

The time evolution of α, β, and γ, which denote the normalized concentrations of P_1, P_2, and P_3, is governed by
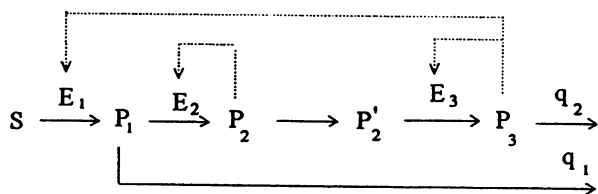
FIGURE 1   The multienzymatic instability-generating reactive system.

the following three delay differential equations:

$$\frac{d\alpha}{dt} = z_1\sigma_1\Phi_1(\mu) - \sigma_2\Phi_2(\alpha, \beta) - q_1\alpha,$$

$$\frac{d\beta}{dt} = z_2\sigma_2\Phi_2(\alpha, \beta) - \sigma_3\Phi_3(\beta, \beta', \mu),$$

$$\frac{d\gamma}{dt} = z_3\sigma_3\Phi_3(\beta, \beta', \mu) - q_2\gamma,$$

Here

$$\Phi_1 = \mu S K_{D3}/(K_3 K_2 + \mu K m_1 K_{D3} + S K_2 + \mu S K_{D3}),$$

$$\Phi_2 = \frac{\alpha(1 + \alpha)(1 + d_1\beta)^2}{L_1(1 + c\alpha)^2 + (1 + \alpha)^2(1 + d_1\beta)^2},$$

$$\Phi_3 = \frac{d_2\beta'(1 + d_2\beta')^3}{L_2(1 + d_3\mu)^4 + (1 + d_2\beta')^4},$$

where

$$\beta' = f(\beta(t - \lambda_1)),$$

$$\mu = h(\gamma(t - \lambda_2)),$$

The various parameters, besides the constant input S and the sink constants q_1 and q_2, are related to the kinetic properties of the enzymes hexokinase, phosphofructokinase, and pyruvate kinase (Table 1; see de la Fuente et al., 1995, for further details).

In the aforementioned reference we studied by means of the functional variables μ and β' the consequences that phasic changes in the oscillations of the concentrations of metabolite ATP and phosphoenolpyruvate have on the behavior of the integral solutions of the dynamical system.

RESULTS

First of all, we have assumed that the system is under constant input.

The calculations of the obtained oscillations have delay times $\lambda_1 = 7$, and $\lambda_2 = 130$. The initial functions present the following simple harmonic oscillation:

$$\alpha_0(t) = 26 + 12 \sin(2\pi/P)$$

$$\beta_0(t) = 12 + 10 \sin(2\pi/P)$$

$$\gamma_0(t) = 7 + 6 \sin(2\pi/P)$$

$$P = 534.$$

The functions of the functional variables are supposed to be linear, with $K = 1$.

$$f(\beta, t - \lambda_1) = K\beta(t - \lambda_1)$$

$$h(\gamma, t - \lambda_2) = K\gamma(t - \lambda_2).$$

We have fixed the remaining parameters as described in our aforementioned reference.

Under these conditions, the numerical integration reveals that the temporal structure of the system presents a stable steady state for $q_2 = 0.11$. For $q_2 = 0.103$, a first Hopf bifurcation generates a periodic behavior (Fig. 2 a). Above $q_2 = 0.099$, a second Hopf bifurcation introduces a new fundamental frequency and the system motion becames quasiperiodic (Fig. 2 b and 3). The torus becomes more complex for $q_2 = 0.096$ (Fig. 2 c). And for $q_2 = 0.095$, complex substructures can be observed, which shows the destruction of the torus (Fig. 2 d) and its replacement by a strange attractor, $q_2 = 0.093$ (Fig. 2 e).

In Fig. 2 we show five time series for different values of q_2, along with their power spectra and the Poincaré sections of each attractor. In that way, the power spectra display a clear transition from one to two fundamental frequencies and then to chaos. We can see in Fig. 2 f a periodic movement with one frequency; in Fig. 2, g–i, a quasiperiodic motion with two incommensurate frecuencies and their linear combinations; and finally chaotic motion in Fig. 2 j. In Fig. 2, h and i, the Poincaré sections show the torus break-up, which develops a strange attractor as predicted by Newhouse, Ruelle, and Takens. Under these conditions, the system presents a route to chaos via quasiperiodicity and does not pass through a cascade of period-doubling bifurcations.

Chaotic dynamics via torus have been described for experimental glycolytic oscillations under a periodic source of glucose (Hess et al., 1984; Markus et al., 1985a; Hess et al., 1990). To simulate these experimental observations, our system was induced to oscillate under the following sinusoidal source of substrate injection: $S = S' + A \sin \omega t$, where S' is the mean input flux, and A and ω are the amplitude and the frequency of the periodic input flux.

TABLE 1   Parameters

Parameter	Values	Parameter	Values
σ_1	0.215 s^{-1}	C	10^{-5}
σ_2	4 s^{-1}	q_1	8×10^{-4}
σ_3	0.76 s^{-1}	S	$2 \times 10^{-3} \text{ M}$
Z_1	2	L_1	6×10^5
Z_2	1.5	L_2	10^3
Z_3	1	K_2	$6.3 \times 10^{-5} \text{ M}$
d_1	0.85	K_3	10^{-4} M
d_2	1	K_{m1}	10^{-4} M
d_3	5×10^{-3}	K_{D3}	$2.9 \times 10^{-5} \text{ M}$

FIGURE 2 Oscillatory behaviors under constant substrate input flux, along with the corresponding Poincaré sections and power spectra showing a transition sequence from periodicity to chaos through quasiperiodicity. (*a*) Periodic behavior. (*b–c*) Quasiperiodic motion. (*d*) Complex quasiperiodic motion with substructures indicating the destruction of the torus. (*e*) Chaotic behavior. The first figure (*a–e*) in each row represents β as a function of time; the second figure is the Poincaré section (α, β plane), and the third (*f–j*) is the power spectrum of the oscillations.
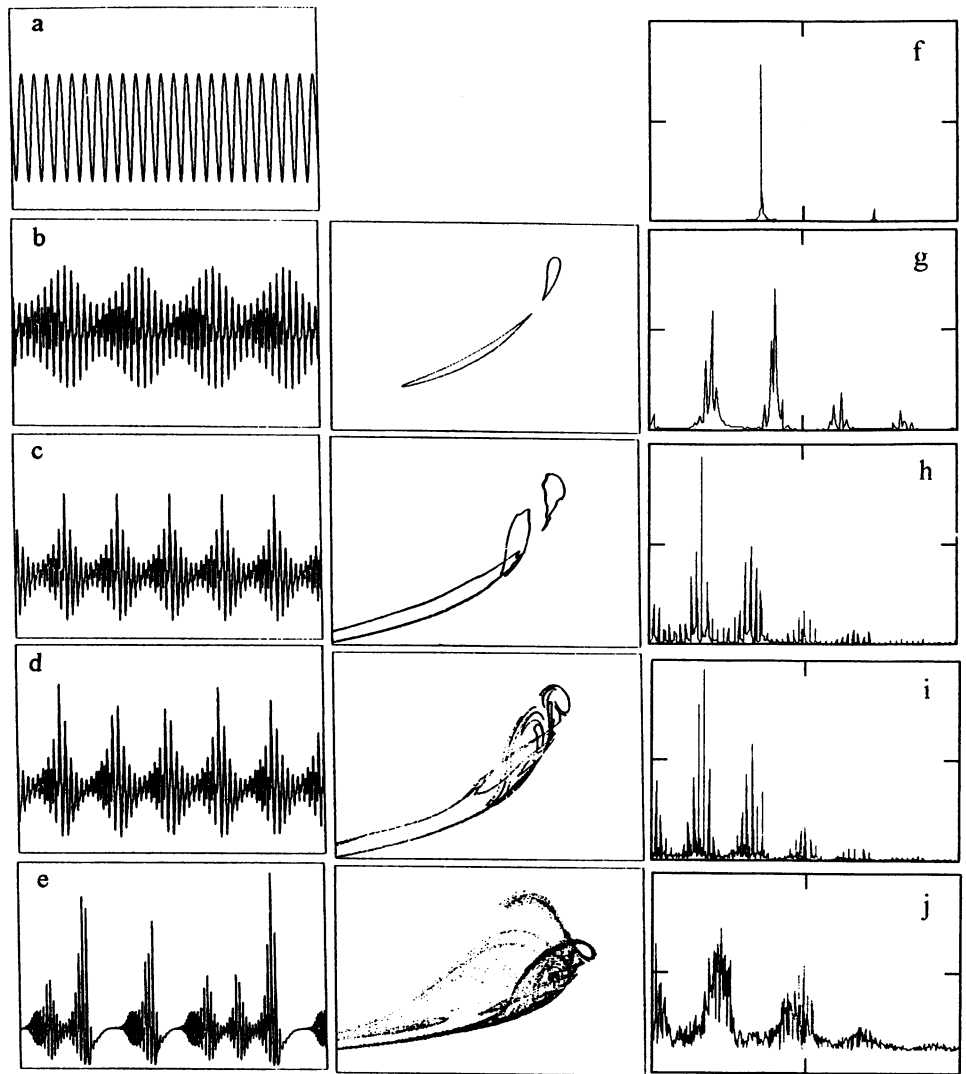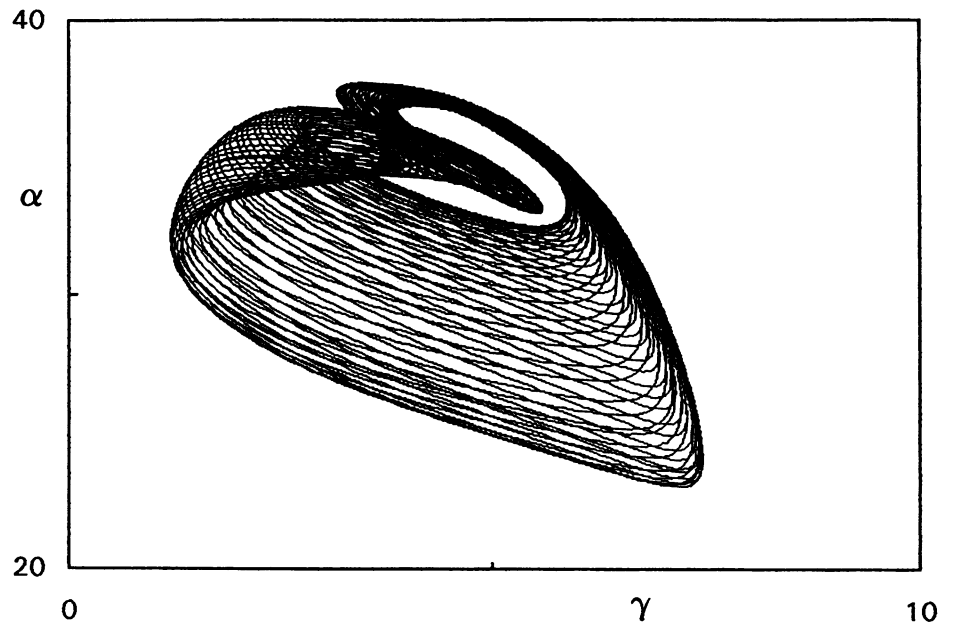
FIGURE 3 Quasiperiodic attractors under constant substrate input flux. Torus for $q^2 = 0.099$ (γ in abscissas and α in ordinates).
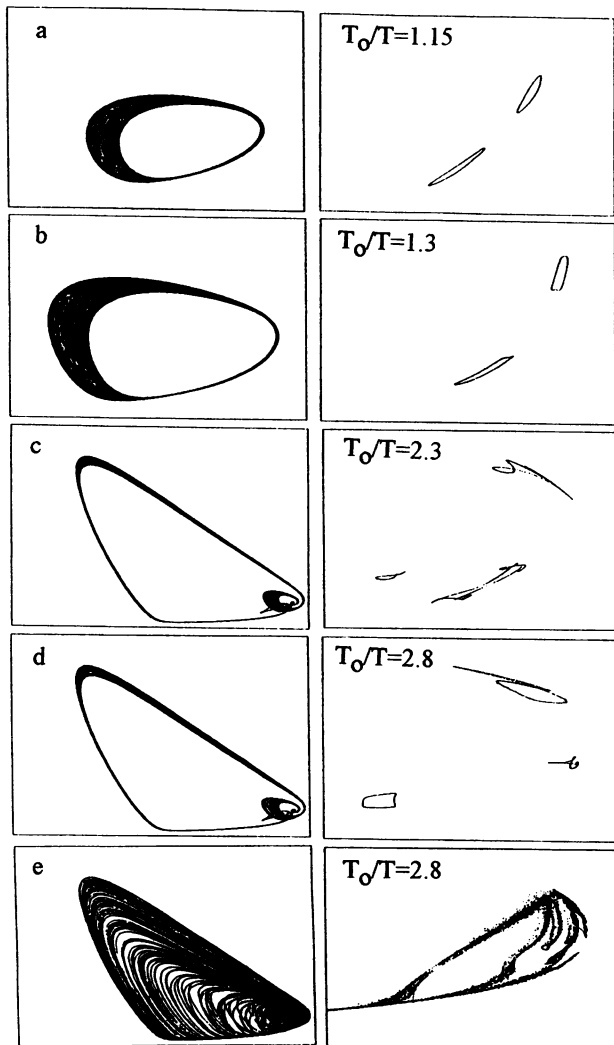
FIGURE 4   Theoretically obtained oscillatory responses of glycolysis under periodic substrate input flux. Five attractors are displayed along with their Poincaré sections. (a–d) Quasiperiodic motion for different values of T_o/T. (e) The quasiperiodic motion in d is replaced by chaos when the system experiments an increment of amplitude of the input flux. The first figure in each row is the attractor in α, β coodinates, and the second figure is the Poincaré section in the α, β plane.

These parameters are normalized (as described in de la Fuente et al., 1995) by dividing them by $K_{m2} = 5 \times 10^{-5}$ M, the Michaelis constant of phosphofructokinase for fructose 6-phosphate (Bartrons et al., 1982).

Assuming $S = 12$ mM/h (Markus et al., 1984), we obtain the normalized input flux $S' = 0.06$ s^{-1}, and the sinusoidal expression for the input is $S = 0.06 + A \sin \omega t$. The values of the other parameters are $\lambda_1 = 7$ and $\lambda_2 = 50$.

Under a periodic input flux condition, the temporal structure of the system reveals a rich variety of time patterns, including quasiperiodic oscillations and chaos.

In the absence of periodic excitation for $S = 0.06$ and $q_2 = 0.1$, the dynamical system has an asymptotically stable solution with a period $T_o = 91.8$ s.

However, under periodic perturbation for $T = 80$ s and $A = 0.057$, the limit cycle experiments a Hopf bifurcation and the oscillation becomes quasiperiodic, with $T_o/T = 1.15$ (Fig. 4 a).

Under constant input flux, the amplitude of oscillations increases. Under these conditions, with sinusoidal glucose input flux, we find quasiperiodic behavior for different values of the period excitation T. Fig. 4, b–d, shows three tori along with their Poincaré sections for the following conditions: b: $q_2 = 0.09$, $T = 84$, $T_o/T = 1.3$; c: $q_2 = 0.08$, $T = 224$, $T_o/T = 2.3$; d: $q_2 = 0.08$, $T = 208$, $T_o/T = 2.8$.

In Table 2 we show quasiperiodic behaviors for different values of A and ω when the sink constant is $q_2 = 0.08$ (A is the amplitude of the input flux, which is normalized here with respect to the mean input flux V_{in}. ω is the frequency of the input flux, which is normalized with respect to the frequency obtained ω_0 at constant input flux.)

Experimentally the chaotic behavior appears when the frequency of the periodic sustrate is between 2 and 3 times the frequency of the autonomous oscillation and for an increment of amplitude of the input flux (Hess et al., 1984; Markus et al., 1985a). According to these results, in our model the emergence of a strange attractor may be observed for the conditions $q_2 = 0.08$ and $T = 208$ ($T_o/T = 2.8$) when the input flux amplitude is incremented from $S = 0.06 + 0.057 \sin \omega t$ (Fig. 4 d) to $S = 0.06 + 0.059 \sin \omega t$ (Fig. 4 e).

DISCUSSION

The dynamical modeling of a dissipative enzymatic system, formed by a system of three functional differential equations with delay, allows us to observe a "complete" transition sequence from a stable steady state to periodicity, quasiperiodicity, and chaos.

The numerical results for our theoretical model show that the described biochemical system may display this complex

TABLE 2   Quasiperiodic behaviors for different values of A and ω

A/V_{in}	ω/ω_0	T (s)	A (s^{-1})
0.05	1.15	507	0.003
0.1	1.3	448	0.006
0.2	1.3	448	0.012
0.1	1.4	416	0.006
0.2	1.4	416	0.012
0.1	1.6	364	0.006
0.1	1.9	307	0.006
0.3	1.9	307	0.018
0.5	1.9	307	0.03
0.1	2.2	265	0.006
0.2	2.2	265	0.012
0.3	2.2	265	0.018
0.1	2.4	243	0.006
0.2	2.4	243	0.012
0.1	2.8	208	0.006
0.3	2.8	208	0.018
0.5	2.8	208	0.03

FIGURE 5 Experimentally predicted excitation diagram of glycolysis under periodic input flux (Hess et al., 1990). A is the amplitude of the input flux, which is normalized with respect to the mean input flux V_{in} (in ordinates). ω_e is the frequency of the input flux, and ω_0 is the frequency obtained at constant input flux (in abcissas). QP, quasiperiodicity; C, chaos. Different quasiperiodic behaviors emerge for $1 < T_0/T < 3$, and the chaos arises for $2.5 < T_0/T < 3$ at high amplitudes of the input flux. Crosses show the values of quasiperiodic behaviors obtained with our model (Table 2).
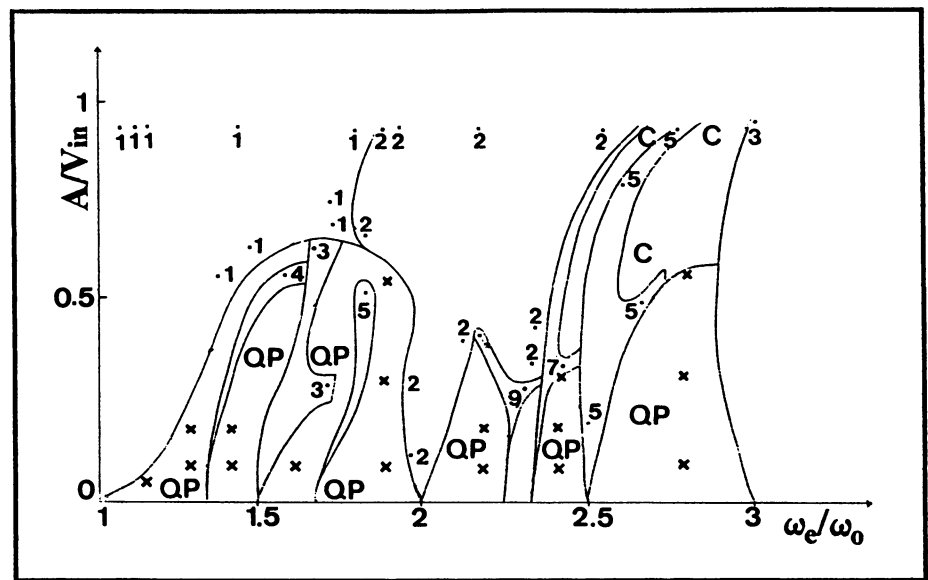
dynamical behavior both under constant substrate input flux and under periodic perturbations.

As we have indicated before, quasiperiodic behavior and chaos in the glycolytic subsystem have been observed by measuring the NADH fluorescence of glycolyzing yeast extracts under sinusoidal glucose input flux (Fig. 5; Hess et al., 1990). In these experiments the response of the system through a periodic source of substrate seems to depend on the magnitude of the period T of the input relative to the autonomous period T_0 of the system at constant input flux. In this way, quasiperiodicity is obtained at low amplitudes of the input flux for $1 < T_0/T < 3$ (Richter and Ross 1980; Markus et al., 1985b; Hess et al., 1990), and chaos is obtained at high amplitudes of the input flux at input frequencies between 2 and 3 times the frequency of the autonomous oscillations for $2.5 < T_0/T < 3$ (Hess et al., 1984, 1990; Markus et al., 1985a).

In our model the different quasiperiodic behaviors emerge for $1 < T_0/T < 3$ (Table 2), and the chaos via quasiperiodicity arises at high amplitude of the input flux for $T_0 = 240$ s, the quotient T_0/T being 2.8.

We can conclude, therefore, that the theoretically obtained quasiperiodic oscillatory responses of glycolysis under periodic substrate input flux are within the range of experimental values (Fig. 5).

Because knowledge of biochemical oscillations is still limited, it is necessary to continue with the theoretical and experimental research in this field.

REFERENCES

Bartrons, R., E. Schaftingen, S. Vissers, and H. Hers. 1982. The stimulation of yeast phosphofructokinase by fructose 2,6-bisphosfate. *FEBS Lett.* 143:137–140.

De la Fuente, M. I., L. Martínez, and J. Veguillas. 1995. Dynamic behavior in glycolytic oscillations with phase shifts. *Biosystems.* 35:1–13.

De la Fuente, M. I., L. Martínez, and J. Veguillas. 1996. Intermittency route to chaos in a biochemical system. *Biosystems.* 39:87–92.

Dubois, M., P. Bergé, and V. Croquette. 1982. Study of the steady convective regimes using Poincaré sections. *J. Physiol. (Paris) Lett.* 43: L295.

Feigenbaum, M. J. 1978. Quantitative universality for a class of nonlinear transformations. *J. Stat. Phys.* 19:25–52.

Hess, B., D. Kuschmitz, and M. Markus. 1984. Dynamics of Biochemical Systems. J. Ricard and A. Cornish-Bowden, editors. Plenum Publishing, New York.

Hess, B., M. Markus, S. C. Müler, and T. Plesser. 1990. From homogeneity towards the anatomy of a chemical spiral. In Spatial Inhomogeneities and Transient Behavior in Chemical Kinetics. P. Gray, G. Nicolis, F. Baras, P. Borckmans, and S. K. Scott, editors. Manchester University Press, Manchester, England.

Hudson, J. L., J. C. Bell, and N. I. Jaeger. 1989. Potentiostatic current oscillations of cobalt electrodes in hydrochloric acid/chromic acid electrolytes. *Ber. Bunsen-Ges. Phys. Chem.* 92:1383–1387.

Markus, M., D. Kuschmitz, and B. Hess. 1984. Chaotic dynamics in yeast glycolysis under periodic substrate input flux. *FEBS Lett.* 172:235–238.

Markus, M., D. Kuschmitz, and B. Hess. 1985a. Properties of strange attractors in yeast glycolysis. *Biophys. Chem.* 22:95.

Markus, M., S. C. Müler, and B. Hess. 1985b. Observation of entrainment quasiperiodicity and chaos in glycolyzing yeast extracts under periodic glucose input. *Ber. Bunsen-Ges. Phys. Chem.* 89:651.

Newhouse, S., D. Ruelle, and F. Takens. 1978. Occurrence of strange axiom-A attractors near quasiperiodic flow on T^m, $m > 3$. *Commun. Math. Phys.* 64:35–44.

Pomeau, Y., and P. Manneville. 1979. Intrinsic Stochasticity in Plasmas. Ed. de Pysique, Orsay.

Richter, P. H., and J. Ross. 1980. Oscillations and efficiency in glycolysis. *Biophys. Chem.* 12:285.

Ruelle, D., and F. Takens. 1971. On the nature of turbulence. *Commun. Math. Phys.* 20:167–172.

Schuster, H. G. 1984. Deterministic Chaos. Physic-Verlag, Weinheim.

Scott, S. 1991. Chemical Chaos. Claredon Press, Oxford.