TESCE, July 2000 PP. 111-156

Chaotic Behavior of Biochemical Systems

Gamal Ibrahim, Hamza Habib and Mohamed F. El-Sherbiny
Department of Basic Engineering Sciences,
Faculty of Engineering,
El-Menoufia University
Shebin El-Kom, Egypt

ABSTRACT

Chaos is the word used to represent aperiodic oscillations , apparently random behavior appearing in a system not subject to stochastic perturbation but entirely governed by a deterministic dynamic law. It is intimately related to periodic oscillation, and periodicity may decompose to chaos when some varying parameter constraining an oscillatory system crosses a critical value. The resulting complex chaotic behavior may be regarded as periodic but with a repetition time approaching infinity. Indeed, one of the major routes from periodicity to chaos involves a repetitive doubling of the period as the bifurcation parameter is varied.

INTRODUCTION

While the classical "phenomenological" definition of the term "chaos" means absence of order and unpredictability, the modern definition of chaos is based on non-linear mathematics whose principles were anticipated during the late 19th century by Poincare , but made mathematically accessible by Lorenz in 1963 in a paper in the Journal of Atmospheric Sciences with the title "Deterministic nonperiodic flow "(2). Today, chaos is defined as unforeseen behavior in a deterministic system or to say it in a more colloquial form : "chaos is apparently lawless behavior totally ruled by (deterministic) laws (3)". In 1987 Skarda and Freeman (4) brought the definition down to one phrase when they described chaos as "pseudorandom noise". Generally the word chaos refers to low-dimensional aperiodic signals , while the term noise is used to describe behavior resulting from very many degrees of freedom (3). The amplitudes and/or periods of the individual cycles of a chaotic behavior look to be random and are unpredictable and irreproducible over an extended period of time. A chaotic system will remain apparently noisy regardless of how well experimental conditions are controlled. However a chaotic behavior results from a quite ordinary deterministic dynamic law and has considerable order related to the presence of a so-called strange attractor that attracts trajectories in the same way as do simpler attractors such as steady state or limit cycle. Order is present because a chaotic waveform stays within a finite region in phase space in the close neighborhood of the strange attractor (3).

The reason why oscillatory behaviors are so common in biochemical biological systems stems from their regulatory properties, which were developed and selected in the course of evolution. Positive as well as negative regulatory feedback provide a source of nonlinearity which, in conjunction with cooperative processes, gives rise to instabilities associated with oscillatory behavior. Such a source of nonlinearity due to regulatory feedback is lacking in chemical systems, which are not subjected to evolutionary pressure . However, this does not rule out the existence of nonlinear, activating or inhibitory processes in chemical kinetics, but these processes are uncommon and remain "gratuitous" as they do not have any physiological role (1). Besides periodic behavior , other, more complex oscillatory phenomena have been identified and increasingly studied in recent years. Among these phenomena are complex periodic oscillations (bursting oscillation) and aperiodic oscillations (chaotic or strange non-chaotic oscillations) .From a mechanistic point of view, two major routes leading to bursting and strange oscillations have been identified (6). These two major types of complex oscillations arise either from the periodic forcing of an oscillatory system, or from the interaction of at least two instability generating mechanisms within the same system. In contrast to the former scenario, which has been followed in many experimental and theoretical studies devoted to chaos in biology, particularly in biochemical and neurobilogical system (7,8) chaos obtained following the second route is autonomous as it occurs in the absence of periodic forcing.

The unpredictability of a chaotic behavior results because trajectories starting from arbitrarily very close initial conditions diverge . The measure of this divergence is the Lyapounov exponent. The fundamental mathematical definition of a chaotic system is one with at least a positive Lyapounov exponent (4,5). This definition and similar measures can be calculated for an experimental waveform, but practical problems often arise that cloud their interpretation. Chaos is thus usually identified in an experimental system or simulation by construction of sorts of maps (e.g. Poincare maps) and by investigation of the route from periodicity to aperiodicity.

Although it is also encountered in mechanical , physical , chemical and electrochemical systems, rhythmic behavior can be viewed as a basic property of living organisms. Oscillations indeed occur at all levels of biological organization (5) , with periods ranging from milliseconds (neurons) to seconds (cardiac cells), minutes (oscillatory enzyme) , hours (pulsatile hormone secretion), 24 h. (Circadian rhythms), weeks (ovarian cycle) and years (circannual rhythms, epidemiological processes and predator-prey interaction in ecology).

I- Oscillations of Biochemical Systems

The number of experimentally observed oscillations in biochemical systems did not significantly change during the decade following 1975, when cyclic AMP (c-AMP) oscillations were observed in the slime mold Dictyostelium discoidens (9) . Some ten years before, around 1965, oscillations were demonstrated in glycolysis, first in intact yeast cells and then in yeast (and later muscle) extracts (10-14) . Around the same time oscillations are also found in the peroxidase reaction (15) and in mitochondria (12, 16). Studies of the latter oscillations were , however, not pursued much beyond their initial characterization oscillation were also found in acetylcholinesterase acetylcholine system (17).

In 1985 (18) , oscillations in intracellular ca^{++} were added to the list of periodic phenomena observed at the cellular level (19-22). These widespread oscillation in cytosolic ca^{++} differ from those involving voltage-dependent membrane conductances in electrically excitable cells.

Recent experimental advances have thrown light on the oscillator which controls the onset of mitosis in eukaryotic cells. Evidence which has accumulated in the last few years points to the existence of a continuous biochemical oscillator underlying the cell-division cycle in embryonic cells (23).

The experimental and theoretical studies of the most important examples of periodic and/or aperiodic behavior in biochemical systems will be discussed in this section.

I-1 The Peroxidase - Oxidase Reaction

The reaction in question is the peroxidase-oxidase (PO) reaction, which is the oxidation of organic electron donors by molecular oxygen, catalyzed by the enzyme horseradish perioxidase when this reaction takes place in a flow system with reduced nicotinamide adenine dinucleotide (NADH) as the reactant, the concentrations of reactants (oxygen and NADH) as well as some enzyme intermediates can be seen to oscillate with periods ranging from several minutes to about an hour, depending on the experimental condition.

Yamazaki and co-workers discovered (15) in 1965 that the perioxidase-catalyzed oxidation of NADH occurs via damped oscillations when oxygen is supplied continuously by bubbling a mixture of oxygen and nitrogen through the reaction mixture. A very similar system, where the bubbling of oxygen through the solution is replaced by diffusion through the gas/liquid interface from a gas head space, was shown to exhibit bistability, i.e. the existence of two simultaneously stable steady states for the same oxygen concentration in the gas phase.(24) . Temporary perturbations in the oxygen concentration in the gas phase could induce reversible switches from one steady state to the other. The bistability phenomenon is thought to be due to inhibition of the enzyme by O_2.

Degen showed(25) somewhat later (1969) that damped oscillations also could be obtained using the substrates dihydroxyfumaric acid and indoleacetic acid instead of

NADH. The oscillations were accompanied by measurable chemiluminescence. The chemiluminescence was ascribed to free-radical intermediates and taken as evidence of the presence of autocatalysis in the reaction mechanism.

Sustained oscillations in the PO reaction were first obtained by Nakamura et al. (26) using NADPH as the substrate; NADPH was regenerated from the oxidized form ($NADP^+$) by glucose-6-phosphate and glucose-6-phosphate dehydrogenase. Sustained oscillations were found only when the modifiers 2,4-dichlorphenol (DCP) and methylene blue (MB) were present, Olsen and Degn later reported sustained oscillations (27) with a constant infusion of NADH, thus demonstrating that glucose-6-phosphate dehydrogenase was unnecessary to sustain the oscillations (See Fig. 1). However, these authors found that the presence of DCP (and, perhaps, MP) were critical. Olsen and Degn (27) in 1978 provided further evidence that the oscillatory behavior is more likely due to autocatalysis than to substrate inhibition by oxygen.

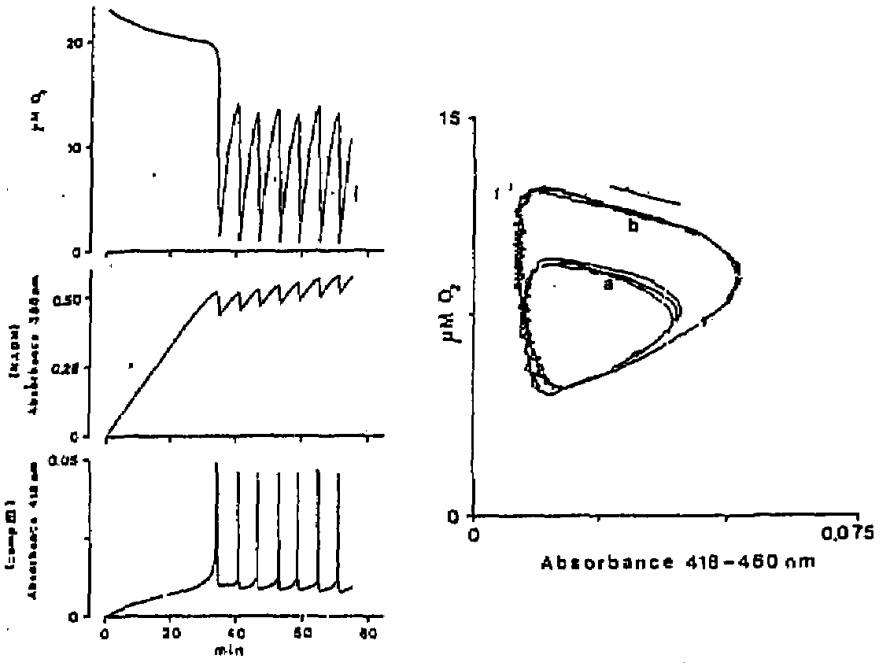


Fig. (1) Oscillatory behavior in the peroxidase-NADH-O_2 reaction [from L.E. Olsen and H. Degn , Biochim. Biophys. Acta , 523 , P 321 (1978)]

Observations of chaos: Olsen and Degn observed in 1977 that the waveform of the PO oscillations depends strongly on the concentration of peroxidase (28). Simple periodic oscillations with period of about 5 min were obtained at enzyme concentrations of about 1 μM, whereas bursting oscillations with periods of up to 60 min were seen at enzyme concentration below 0.5 μM. The oscillations were aperiodic and irregular at enzyme concentration slightly above 0.5 μM (Fig. 2). A smooth curve fitted to the points in a next-amplitude map of the data was used to carry out symbolic dynamics, a period-three cycle was found for certain initial conditions. The theorem of Li and Yorke (29) that the existence of a period-three oscillation implies chaos was then used to argue that the irregular oscillations were in fact chaotic. This observation was made less than a year after publication of the pioneering paper by Rossler (30) suggesting that chaos might be found in chemical reactions. Schmiz, Graziani and Hudson reported observations of chaos in the BZ reaction (31) only a few months after its observation in the PO reaction.

The PO reaction remains as of this writing the only enzyme reaction shown to behave chaotically without the imposition of periodic forcing. Markus, Kuschmitz and Hess (32) have shown that the glycolytic reaction exhibits a chaotic response when the supply of glucose is periodic. However, although the existence of autonomous chaos in glycolysis has been predicted theoretically (33), there is still no experimental verification.

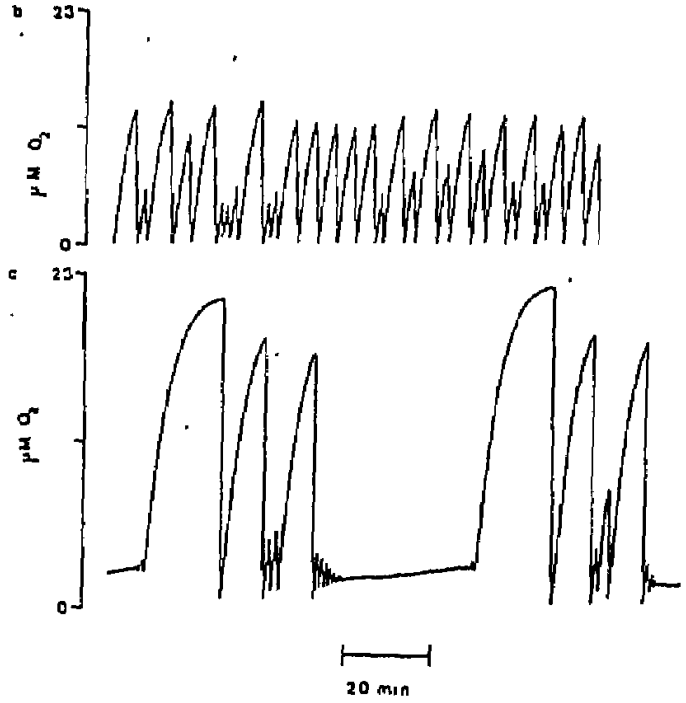


Fig. (2) Chaos in the PO reaction . Experimental conditions have been found in reference (28)

Application of the theorem of Li and Yorke (29) to the next-amplitude maps of the aperiodic oscillations in the PO reaction remained for 15 years the only experimental evidence for chaos in this reaction. This experimental evidence was supported by numerical simulations which yielded next-amplitude maps that were very similar to the experimental maps. Recent experiments by Geest et al. (34) have demonstrated that chaos in the PO reaction arises by the well-known period-doubling route as the concentration of DCP is varied over a critical range (see Fig.3) . A similar period-doubling route to chaos has been predicted from simulations with a detailed model of the PO reaction for variations of the enzyme concentration (35). This prediction has yet to be verified experimentally; however,the Lyapounov exponent and fractal dimension were computed in a further study by Geest et al. (1) for the experimental data and compared with those obtained from models of the reaction. The Lyapounov exponent measures the average rate at which two initially close trajectories converge or diverge. A positive exponent implies chaos, and hence the Lyapounov exponents computed from the time series by Geest et al. (34) suggest chaotic motion.

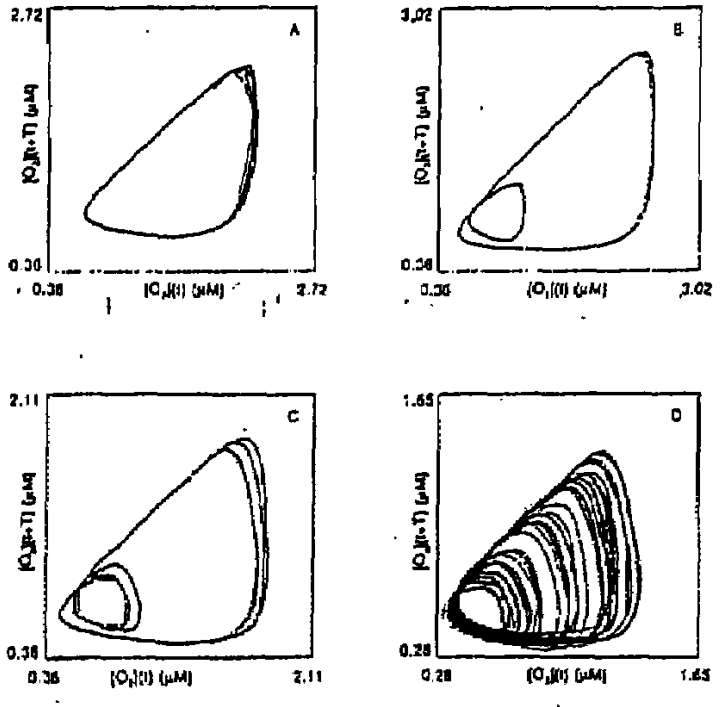


Fig. (3) Two- dimensional projections of phase plots in the oxygen concentration as DCP is varied showing : a) period 1 ; b) period 2 ; c) period 4 ; d) chaos.. Ref.(34)

The fractal dimension measures the information needed to specify the position of a point on an attractor to within a given accuracy and hence, in some sense, expresses the complexity of the motion. Simple types of motion have low-integer dimensions. For example, the dimension of a steady state is zero whereas a limit cycle oscillation has a dimension of one. Chaotic motions usually have finite but non-integer dimensions, also known as fractal dimensions. Dimensions in the range of 2.45 to 2.7 obtained for the experimental data are indicative of chaotic motion and are also strikingly in accord with theoretical values previously predicted from a simple model of the PO reaction . These recent experimental results thus confirm that the irregular oscillations observed by Olsen and Degn in 1977 are indeed chaotic.

Recent experiments at Stanford University (36) have revealed the existence of quasiperiodic oscillations in the PO reaction using the same experimental configuration as Olsen and Degn; whether or not this quasiperiodicity is associated with a different route to chaos is presently unknown. Both period doubling and quasiperiodicity are well-known routes to chaos, and both have been found to be associated with chaos in models of the PO reaction.

Theoretical understanding of the kinetics of the PO reaction is quite detailed. Much of this study has been based upon the early work of Olsen and Degn (27) who proposed a four-variable model composed of two coupled autocatalytic cycles. Further computer simulations and theoretical explanations of the behavior of this model were given by Degn, Olsen and Perram (37), so this model has come to be known as the DOP model. Degn et al., reported in their 1979 paper that the Dop model seemed to be incapable of supporting chaotic behavior. Later work by Larter et al. (38, 39) showed that chaos could, in fact, be found within narrow ranges of parameter values. Olsen (40) suggested a slightly modified model prior to this discovery to explain the existence of chaos in the PO reaction.

Several groups simultaneously were carrying out computer simulation studies of detailed models based on the twenty or so possible reaction steps which may occur in the PO reaction. Yokota and Yamazak (41) proposed a detailed mechanism and found some agreement between simulations based on it and the induction kinetics, but never reported observing oscillatory behavior. Fed'kina, Brounkova and Ataullakhanov (42) studied a similar mechanism by reducing it to a subset of approximate rate equations for the two species H_2O_2 and NAD. Oscillatory behavior was found in this (greatly reduced) subsystem. Using a formalism known as stoichiometric network analysis, Aguda and Clarke (43) extracted a subset of ten crucial steps from the twenty possible reactions and showed that these ten steps are sufficient to explain the bistability and damped oscillations. Later studies (44) showed that the ten-step mechanism also can explain the existence of sustained oscillations. More recently, the addition of two more steps (35) has led to the discovery of chaotic behavior in the latest detailed model. It is interesting that certain details of the experimental observations which were not reproduced by the four-variable models are, in fact, seen in simulations with the detailed mechanisms. A detailed description of the modeling and simulation efforts are found in reference (1) in the article written by Larter, Olsen, Steinmetz and Geest.

Computational studies of a detailed model by Aguda and Larter (44) led them to predict that under certain conditions, the PO reaction might possess coexisting stable states, one oscillatory and the other stationary. This type of bistability between a limit cycle and a steady state also is known as a "hard excitation". Aguda, Hofmann Frisch and Olsen (45) confirmed this prediction experimentally. Specifically, it had been predicted that a perturbation of the steady state by a sudden cut-off of the oxygen flow would lead to a transition from the steady state to the coexisting oscillatory state. Furthermore, a transition from the oscillatory state to the steady state could be induced by perturbing the reaction with a spike of H_2O_2. The transition from one state to the other was found to be reversible, as predicted by the simulations.

Some quantitative disagreement between experimental observations and the computational predictions were noted, however. Experimentally, the steady state was found to correspond to an oxygen concentration midway between the minimum and maximum O_2 concentrations of the oscillatory state, as opposed to the simulations which predicted the steady state O_2 concentration to be lower than the minimum O_2 level of the oscillatory state. The computational study further predicted that the observed bistability was due to an S-shaped steady-state curve in which the upper branch undergoes a Hopf bifurcation. The experimental results seem to suggest that if an S-shaped steady-state curve underlies the observed dynamics, then $[O_2]$ is not the variable which is multiple valued. Rather, the S-shaped curve might correspond to multiple steady state values in one of the other species, each of which yields the same, or nearly the same, O_2 concentration. An alternative and equally valid explanation of the experimental results would involve a stable limit cycle surrounding an unstable limit cycle, which itself surrounds a stable steady state, i.e, bistability would exist between a locally stable steady state and a surrounding stable limit cycle. No evidence for this latter scenario was found in the computational studies, but it cannot be ruled out experimentally.

Lazar and Ross have carried out studies (46, 47) of the effect of periodic perturbations of the oxygen inlet flow on the PO reaction and have found that these perturbations affect the overall reaction rate and the free-energy dissipation. The system studied was similar to that used by Nakamura et al. (26) in that a second enzyme, glucose-6-phosphate dehydrogenase, was used to regenerate NADH from NAD^+. (The glucose-6-phosphate dehydrogenase used by Lazar and Ross (46, 47) was different from the enzyme used by Nakamura et al. (26) in that it reduces NAD^+ and $NADP^+$ equally well). The rate of reaction in these experiments was determined from the slope of the [NADH] time series while the free energy of reaction was calculated from the defining equation and the measured concentrations of substrates. The perturbation frequency was varied, and it was found that while all frequencies lower the dissipation (and hence increase the efficiency) the smallest effect occurred for frequencies near the autonomous frequency of the reaction. This latter observation was attributed to the fact that a perturbation at the autonomous frequency increases the free energy but lowers the reaction rate, and that these two effects compensate for each other, thus causing the dissipation to remain nearly unchanged.

One aspect of the experimental investigation oscillations and chaos in the PO reaction that is still not well understood is the role which the additives 2.4-

dichlorophenol (DCP) and methylene blue (MB) play in the oscillatory mechanism (48). Olsen and Degn (27) found that sustained oscillations in the PO reaction can be obtained with only DCP present. However, these oscillations were not stable over long times unless MB also was present.

Recently Seveik and Dunford (49) published kinetic studies of the catalysis of NADH oxidation by MB itself. They pointed out that a related reaction, the MB catalyzed oxidation of sodium sulfide, was found to be oscillatory by Burger and Field (50) in this latter reaction the MB catalysis was thought to occur autocatalytically. Seveik and Dunford also pointed out that the flow rate of O_2 into the reaction mixture is a critical parameter in both the Burger and Field experiment and in the experimental configuration leading to oscillations in the PO reaction. Their studies of MB catalysis of NADH oxidation were carried out oxygen flow rates outside the range of values where oscillations are to be expected.

The Seveik-Dunford results raise the issue of whether it is possible for MB to play a role in the PO mechanism as a second catalyst for the oxidation of NADH (peroxidase is the first catalyst). Evidence against this possibility is given by the experiments of Degn (25) which showed that nearly sustained oscillations may be obtained without MB when substrates other than NADH, such as dihydroxyfumaric acid and indoleacetic acid, are used. However, chaotic oscillations were never observed with these latter substrates; hence MB may play a role in the more complex behavior observed in the PO reaction., If the role of MB in the PO reaction is to provide an additional autocatalytic route to oxidation of the substrate (as it does in the Burger-Field reaction), this could explain the existence of chaos in the presence of MB . More experiments are needed to verify these speculations.

Study of the PO reaction has been undertaken using both detailed theoretical and computational investigations as well as some experimental approaches. However, the experimental study lags, by far, the theoretical and computational studies, and the time is now ripe to carry out a new round of detailed experimental investigations. Achieving a higher degree of understanding of this reaction is important since the PO reaction is only the second known example of a homogeneous chemical oscillator that undergoes a well-characterized transition to chaos [BZ reaction is the first example ('6)].
Some of the remaining questions involving this reaction focus on the details of the mechanism involved with the generation of regular and chaotic oscillations. The question of the roles played by the various enzyme species remain unconfirmed . Recent theoretical studies of possible detailed mechanisms bring us closer to answering these questions. Other unsolved problems involve the role of the critical additives methylene blue (MB) and dichlorophenol (DCP) . From dynamical point of view, the origin of the oscillations and the route to chaos have only begun to be studied. Further extensive investigation of this system is necessary to reach the possible maximum understanding of the nonlinear dynamics of the PO reaction.

I-2 Glycolytic Oscillations

To this date, glycolytic oscillations remain the prototype of periodic behavior originating from the regulation of an enzyme reaction. In yeast, glycolytic oscillations occur with a period of 5 to 10 minutes when a substrate such glucose or fructose is injected at an appropriate rate(66) . oscillations also occur in muscle extracts with a somewhat longer period of the order of 20 minutes (67, 68).

It was demonstrated soon after this observation that glycolytic oscillations originate from the reaction catalyzed by phosphofructokinase (PFK) , a key regulatory enzyme controlling the glycolytic flux (10-12) . The PFK oscillations stem from the peculair regulation of this allosteric enzyme: PFK is indeed activated by ADP, one of the reaction products. In muscle , autocatalysis is primarily exerted by the other product of the reaction , fructose-1,6-P_2 (67, 68).

The most conspicuous property of glycolytic oscillations is their control by the substrate injection rate. The range of constant input rates producing oscillations is bounded by two critical values (66) . The period of the oscillations diminishes while the amplitude passes through a maximum as the input rate increases from the first up to the second critical value.

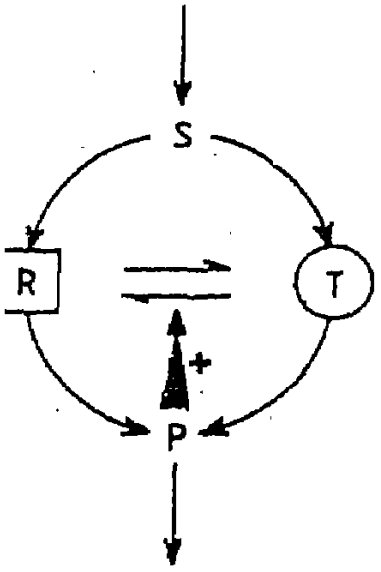


Fig. (4) Schematic representation of a product-activated, allosteric enzyme reaction. The substrate S_1 injected at a constant rate, binds to the states R and T of the enzyme and is subsequently transformed into product P. The product is removed at a rate proportional to its concentration and also promotes the transition from the less reactive (T) to the more reactive (R) enzyme state.The enzyme contains multiple subunits which undergo the conformational transition between the R and T states in a concerted manner.

Following the proposal of phenomenological, enzymatic models or glycolytic oscillations based on the positive feedback exerted by the reaction product, (69, 70) a model taking explicitly into account the allosteric nature of the product-activated enzyme was analyzed for the PFK reaction (6, 71). The model (see Fig. 4) is governed by two differential equations which describe the time evolution of the substrate, α and product γ normalized concentrations in continuously stirred yeast extracts:

$$\frac{d\alpha}{dt} = \nu - \sigma\phi \qquad (1)$$

$$\frac{d\gamma}{dt} = q\sigma\phi - k\gamma \qquad (2)$$

with

$$\phi = \frac{\alpha(1+\alpha)(1+\gamma)^2}{L+(1+\alpha)^2(1+\gamma)^2} \qquad (3)$$

In these equations, ν and σ are the normalized substrate input and maximum enzyme reaction rate, respectively; q is a dimensionless parameter , and L is the allosteric constant closely related to the degree of cooperatively of allosteric interactions between enzyme subunits. Equation 3 gives the simplest form of rate function for the product-activated allosteric enzyme, assuming that the enzyme consists of two identical subunits obeying the concerted transition model of Monod, Wyman and Changeux (72).

Linear stability analysis of Eqs 1-3 indicates that (71) for appropriate values of the other parameters, the unique steady state becomes unstable when the substrate input rate is between two critical values :

$$\nu_{1c} \leq \nu \leq \nu_{2c}$$

This simple model can exhibit simple periodic phenomena : Limit cycles simple bistability (coexistence of stable limit cycle with stable steady state). Introducing a third dimension to this system makes the obtaining of complex dynamic behavior : bursting, chaos, birhythmicity, even trirhythmicity possible. This will be considered in section II.

I-3 Intracellular Ca^{2+} Oscillations

In a large variety of cells, stimulation by an external signal such as a hormone or a neurotransmitter triggers a train of cytosolic Ca^{2+} spikes (9,22). The period of these oscillations generally ranges from seconds (cardiac cells) to minutes (endothelial cells, fibroblasts, or hepatocytes to cite but a few examples). The frequency of Ca^{2+} oscillations rises with the degree of stimulation. Below a critical magnitude of stimulation, cytosolic Ca^{2+} settle at a low steady-state level; above a second, higher critical value of the external stimulus, a high steady-state level of cytosolic Ca^{2+} is established . In some cells such as cardiac myocytes or pituitary cells, Ca^{2+} oscillations can occur spontaneously in the absence of stimulation (6).

While the number of experimental studies of Ca^{2+} oscillations has rapidly increased in the last few years, interest also has been extended to the spatial aspects of Ca^{2+}

signalling (73) . The propagation of Ca^{2+} waves had long been observed in amphibian eggs after fertilization (74) . In hepatocytes (75) and endothelial cells (76), a link between Ca^{2+} oscillations and the wave propagation of Ca^{2+} signals has since been established. The same link has been demonstrated in cardiac myocytes where Ca^{2+} waves propagate as a sharp band along the cell, at a rate (77) of the order of 100 ms^{-1}. In contrast, Ca^{2+} waves in hepatocytes and endothelial cells propagate as "tides" (20) with a progressive increase in Ca^{2+} all over the cell, at a rate (75, 76) close to 20 ms^{-1}.

The mechanism of Ca^{2+} oscillations and Ca^{2+} wave propagation involves the synthesis of inositol 1,4,5-trisphosphate (IP_3) (19, 73). The level of this intracellular messenger increases after stimulation, owing to the activation of phosphoinositidase C. The role of IP_3 is to mobilize Ca^{2+} from an intracellular store. Models for Ca^{2+} oscillations primarily differ according to whether or not they rely on concomitant oscillations in IP_3 (78).

In the model proposed by Meyer and Strycer (79) , oscillations originate from the elevation of the cytosolic Ca^{2+} level by IP_3 and from the activation of IP_3 synthesis by Ca^{2+}. Such a cross-feedback loop results in a global process of self-amplification; Ca^{2+} oscillations are necessarily accompanied by a periodic variation in IP_3. Extensions of the original version of this model have been proposed (21).

A second class of models relies on the process of Ca^{2+} - induced Ca^{2+} release (CICR) to account for Ca^{2+} oscillation (78, 80, 81). Here (see Fig. 5) the rise in IP_3 triggers a constant release of Ca^{2+} from an IP_3 sensitive store into the cytosol. Cytosolic Ca^{2+} is transported into a second store, insensitive to IP_3 from which it is eventually discharged in a process activated by cytosolic Ca^{2+}; the latter CICR process has been demonstrated only in cardiac and muscle cells but some evidence for its occurrence in other cell types has been obtained .

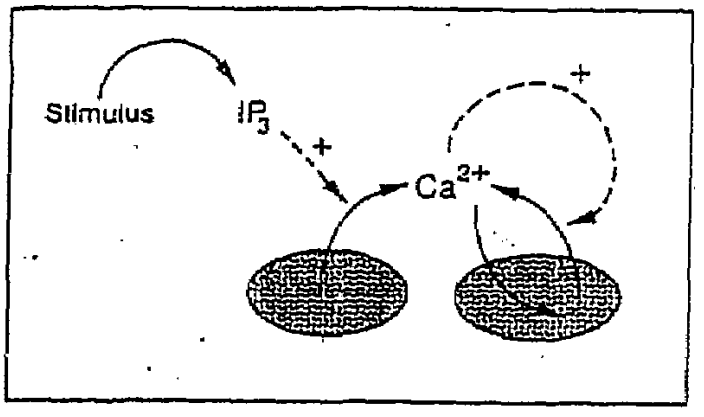


Fig. (5) Schematic representation of the model for Ca^{2+} oscillations based an Ca^{2+} induced Ca^{2+} release . An extracellular stimulus elicits the synthesis of inositol 1,4,5- trisphosphate (IP_3 which mobilizes Ca^{2+} store from which it is released in a process activated by cytosolic Ca^{2+} . The latter regulation incapable of producing sustained Ca^{2+} oscillations (Redrawn from ref. 78).

The model based on CICR, in its simplest form, is governed by two differential equations which describe the time evolution of cytosolic Ca^{2+} (Z), and

$$\frac{dZ}{dt} = v_o + v_1\beta - v_2 + v_3 - kZ \qquad (4)$$

$$\frac{dY}{dt} = v_2 - v_3 \qquad (5)$$

with

$$v_2 = V_2 \frac{Z^n}{(K_2^n + Z^n)}, v_3 = V_3 \frac{Y^m}{(K_R^m + Y^m)} \cdot \frac{Z^p}{(K_A^p + Z^p)} \qquad (6)$$

In the above equations, V_2, and V_3 denote the maximum rates of Ca^{2+} pumping into the IP_3 , insensitive intracellular store and of Ca^{2+} release into the cytosol. The rates n_2 and n_3 have been written so as to allow for positive cooperatively in pumping and release, as well as in the activation of the latter process by cytosolic calcium; K_2 , K_R and K_A denote the threshold constants for these processes, while n, mm, and p represent the Hill coefficients characterizing their degree of cooperatively (n, m, p≥ 1). The threshold constant K_R and concentration Y are both defined with respect to the total intracellular volume.

One of the most salient results of the model based on the CICR mechanism is represented in Fig.6 . There, the temporal evolution of cytosolic Ca^{2+} is shown at different values of the parameter β associated with increasing levels of stimulation. In the absence of stimulation, a stable steady state is established, corresponding to a low level of cytosolic Ca^{2+} (panel A). Upon increasing the value of β , the steady state becomes unstable and oscillations appear, with a frequency that rises with the degree of stimulation (panels B and C). Finally , above a critical degree of stimulation, oscillations disappear and the system evolves towards a stable steady state corresponding to a high level of cytosolic Ca^{2+} (panel D). Similar results are obtained upon raising the level of extracelluular Ca^{2+} (80,81) . The sequence of dynamic behavior predicted by the model in response to increasing levels of stimulus is observed in many cell types (19-22).

In some cells it appears that the distinction between the two pools of Ca^{2+} , one sensitive to IP_3 , and the other to Ca^{2+} , is not so clear-cut, in these cells, indeed, Ca^{2+} and IP_3 behave as co-agonists for the induction of Ca^{2+} release (82). The analysis of such a dual regulation of the Ca^{2+} channel indicates that sustained oscillations of cytosolic Ca^{2+} may still occur in a one-pool model in these conditions (83).

The model based on CICR can be applied to Ca^{2+} wave propagation once the diffusion of cytosolic Ca^{2+} is taken into account. The analysis shows (78) that waves similar to those observed in cardiac cells or in hepatocytes and endothelial cells can occur, with the observed rates, depending on whether the period of Ca^{2+} oscillations is of the order of seconds, as in cardiac cells , or minutes, as in the latter types of cells. The model also has been used to generate spatial patterns similar to those observed in oocytes (84).

Most Ca^{2+} oscillations observed in the experiments are of the simple rather than complex periodic (or chaotic) type. In some cells, however, stimulation by certain agonists triggers a train of Ca^{2+} spikes riding on a slower oscillation (see, e.g., ref 73) , such behavior, reminiscent of bursting , suggests the interplay between at least two

regulatory mechanisms. It is likely that the slower oscillation involves the variation of IP_3 while the faster spikes originate from CICR.

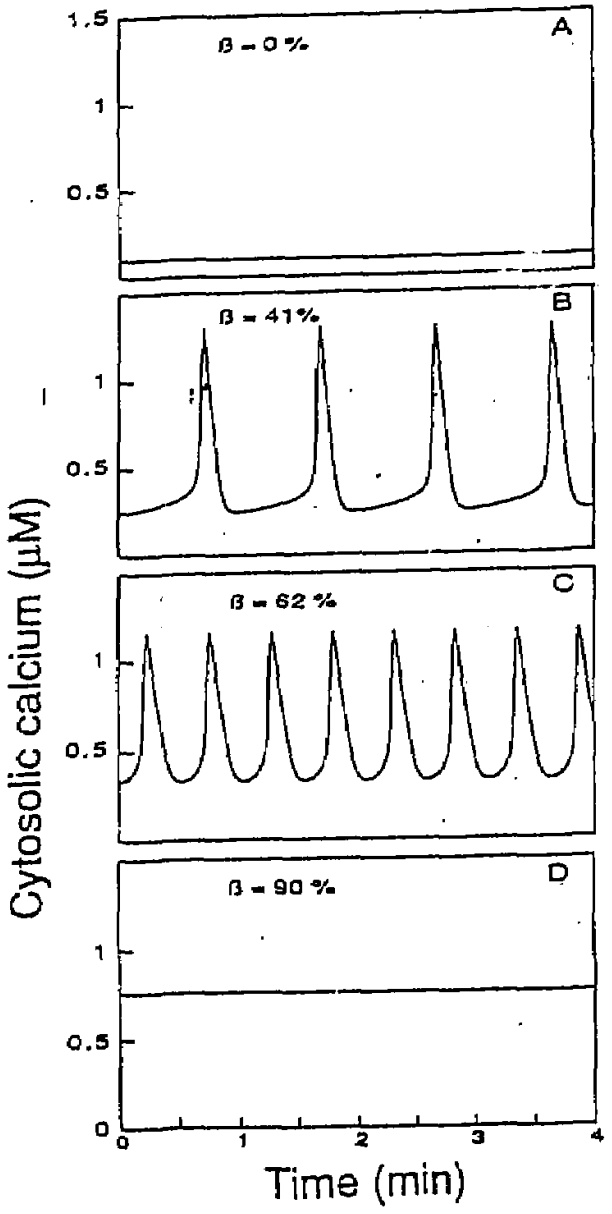


Fig. (6) Sustained oscillations in cytosolic Ca^{2+} generated by means of Eqs. 4-6 for increasing values of b measuring the stimulation level in the model based on Ca^{2+} - induced Ca^{2+} release (ref. 78)

I-4 The Mitotic Oscillator

Besides Ca^{2+} oscillations, the most important oscillatory process discovered in cell biology during the last decade is the biochemical oscillator controlling the onset of mitosis in eukaryotic cells. Advances in the molecular identification of the mitotic control system originate, primarily, from biochemical studies performed in frog, sea urchin and starfish embryos on the one hand (23) and in yeast on the other hand (85). Oscillations in yeasts should be discussed in section I-5.

While the experimental evidence points to the existence of a continuous biochemical oscillator driving the onset of mitosis in rapidly dividing embryonic cells with a periodicity of the order of 30 min (23), the situation is more complex in yeast and somatic cells (85). There, additional feedback processes exist; the mechanism regulating the onset of mitosis is blocked until a sufficient cell size is reached or processes such as DNA replication or mitotic spindle formation are completed. Evidence for a continuous mitotic oscillator has also been obtained in *Drosophila* where the 13 first nuclear divisions occur with a period close to 8 min.

In embryonic cells, the cell-division cycle is driven by a protein named cyclin (23, 86). The progressive accumulation of cyclin leads, through a cascade of biochemical reactions, to the activation of an enzyme called cdc2 kinase (the name originates from genetic studies in yeast where a number of cell division cycle-or cdc-mutations have been characterized) . The periodic activation of cdc2 kinase initiates in turn the various events associated with cell division, such as breakdown of the nuclear envelope, chromosome condensation or spindle assembly.

The regulation of cdc2 kinase is achieved by a cascade of phosphorylation-dephosphorylation reactions (87-89). Several models have recently been proposed for the mitoticoscillator. Most of them rely (90-92) on the nonlinear, autocatalytic activation of cdc2 kinase for which some experimental evidence exists. However, the analysis of a minimal cascade model (93) based on experimental observations (94) shows that autocatalysis is not required for producing oscillations in cdc2 kinase activity. In this model (Fig.7) cyclin is synthesized at a constant rate and activates enzyme E_1 (a protein phosphatase, product of the gene cdc25) which dephosphorylates the inactive form of cdc2 kinase (M^+) and thereby brings it into the active form (M); a protein kinase (E_2) reverts this activation step. The active form of cdc2 kinase in turn phosphorylates the inactive form of a cyclin protease (X^+) and thereby brings it into its active site (X); this activation step is reversed by a phosphatase (E^4). The protease X degrades cyclin, and this step triggers the inactivation of cdc2 kinase. It is possible, and even likely, that the activation of cyclin protease by cdc2 kinase involves additional phosphorylation-dephosphorylation cycles (94), such enlargement of the cascade would, however, not significantly alter its dynamic behavior.

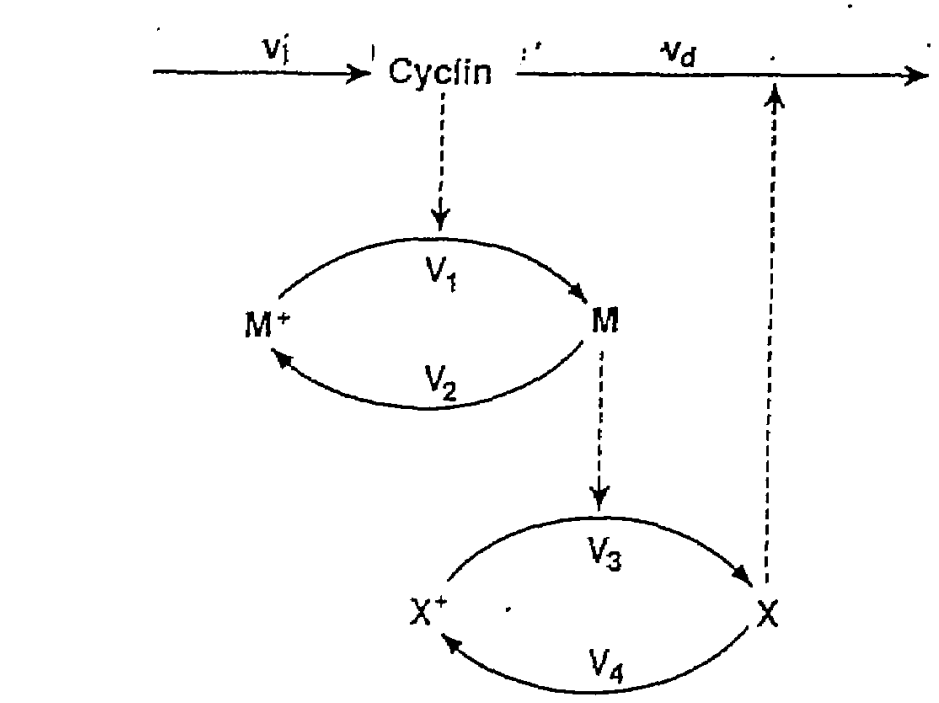


Fig.(7) Minimal cascade model for the mitotic oscillator.. The model incorporates cyclin synthesis, activation of cdc2 kinase by cyclin through a dephosphoylation-phosphorylation cycle, activation of cyclin protease through a second phosphorylation-dephosphoylation cycle involving cdc2 kinase, and destruction of cyctin by the active form of protease X (redrawn from ref. 93).

It has been suggested (23, 94) that the activation of cdc2 kinase by cyclin and the activation of cyclin degradation by cdc2 kinase constitute a negative feedback loop which may result in sustained oscillations. The analysis of the model of Fig.7 shows that this conjecture holds, provided that thresholds exist in the dependence of cdc2 kinase activation on cyclin, and in the activation of cyclin protease by cdc2 kinase (93).

The model of Fig.7 is governed by a set of three differential equations describing the time evolution of cyclin concentration (C), the fraction of active cdc2 kinase (M), and the fraction of active cyclin protease (X).

$$\frac{dC}{dt} = v_i - v_d X \frac{C}{K_d + C} - k_d C$$

$$\frac{dM}{dt} = V_1 \frac{(1-M)}{K_1 + (1-M)} - V_2 \frac{M}{K_2 + M} \qquad (7,8)$$

$$\frac{dX}{dt} = V_3 \frac{(1-M)}{K_3 + (1-X)} - V_4 \frac{X}{K_4 + X} \qquad (9)$$

with,

$$V_1 = \frac{C}{K_e + C} V_{M1}\ ,\ V_3 = M V_{M3} \qquad (10)$$

Parameters V_i and K_i denote the maximum rate and normalized Michaelis constants of enzyme E_i (I = 1, ..., 4); v_i and v_d denote the input rate and maximum rate of cyclin degradation by protease X, while constant k_d relates to a relatively negligible, nonspecific cyclin degradation. It is noteworthy that the only type of nonlinearity in Eqs. 7-10 is of the Michaelian type. These equations nevertheless admit periodic solutions over a wide range of parameter values, once thresholds occur in the activation of M by C and of X by M . A typical example of oscillatory behavior is shown in Fig.8

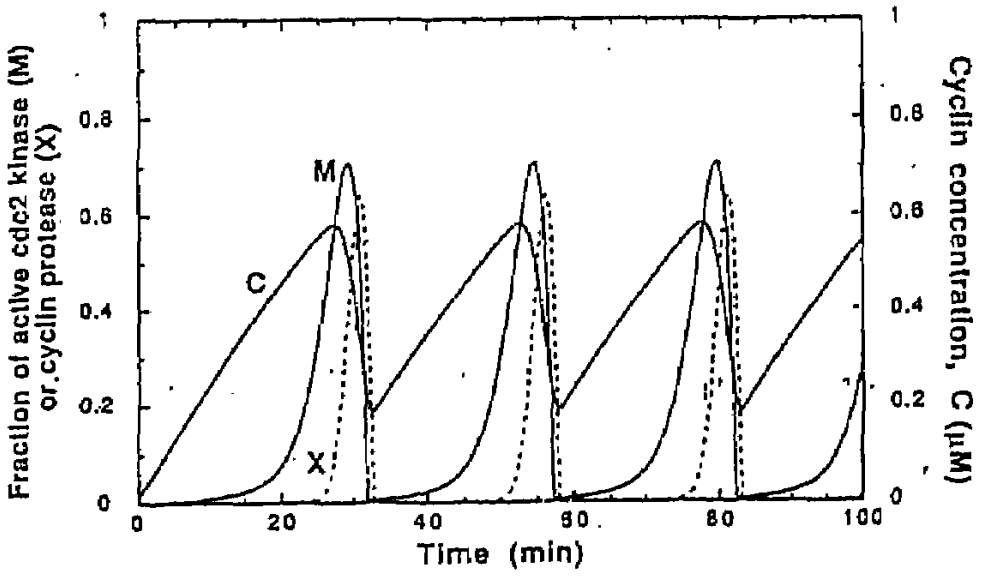


Fig. (8) Sustained oscillations generated by means of Eqs. 7-10 in the minimal cascade model for the mitotic oscillator , schematized in Fig. (7) . [ref. 93]

It can be seen that the progressive rise in cyclin (C) leads to the abrupt activation of cdc2 kinase (M), the latter brings about a sharp rise in cyclin protease (X) after a short delay, and the latter event triggers the rapid decline in cyclin. As a result M drops precipitously, followed by a rapid decrease in X. When the rate of cyclin synthesis exceeds its rate of degradation, the level of cyclin may rise again and a new cycle of oscillations begins.

Although based on a number of simplifying assumptions, such as disregarding the formation of complexes between cyclin and cdc2 kinase and the effect of a second phosphorylation-dephosphorylation reaction involved in cdc2 kinase regulation, the model described by Eqs. 7-10 nevertheless shows that the negative feedback loop built in the cascade suffices, in principle, to produce sustained oscillation. The model indicates the importance of time delays in generating limit cycle behavior; these delays are not introduced in an ad hoc manner into Eq. 7 but result from the existence of thresholds in the activation curves of cdc2 kinase and cyclin protease (93).

Autocatalysis can be incorporated into the cascade model of Fig.8 by assuming that the phosphatase E_1 is itself activated through phosphorylation by cdc2 kinase. The analysis indicates that such an autocatalytic regulation, although not essential for oscillations, renders them more abrupt and enlarges the domain of instability in parameter space.

As the experimental study of the cell cycle is undergoing rapid developments, further insights into the molecular mechanism of the mitotic oscillator can be expected in the near future. It should be noted that the current evidence in this biochemical system points to the occurrence of simple periodic behavior rather than more complex oscillatory phenomena (6).

I-5 Oscillating fermenters

Continuous cultures of Saccharomyces cerevisiae and Zymononas mobilis, two important industrial microorganisms have long been known to exhibit spontaneous oscillatory behavior for some range of the bioreactor dilution rates (95-97). The occurrence of these oscillations adversely affects the optimization and control of the operation of the biofermenter. A number of experimental and theoretical studies are reported in the literature on the causes and means of to eliminate this irregular behavior. One important goal of the experimental studies is the determination of whether these oscillations are inherent in microbial cultures or merely caused by undesired variation in the controlled operating parameters such as pH level, dissolved oxygen levels or other process variables. Parulekar et al.(98) and Porro et al. (99) for instance examined the occurrence of spontaneous oscillations in continuous cultures of the budding yeast and found that the periodic regimes in the chemostat are determined by the dilution rate and the dissolved oxygen concentration (see Fig. 9). Munch et al. (100) also showed that the cell cycle is fundamental in predicting the dynamic behavior of continuous cultures of the microorganism.

Chemostat cultures Z.mobilis, showing sustained oscillations in biomass, product and substrate are also reported in the literature (97, 101). Jobses et al. (102) for instance

reported experimental oscillations in continuous cultures of the microorganism at high ethanol concentration , and suggested means to eliminate them (see controlling chaos section III). Sustained oscillations in microbial cultures have been observed even when the feed conditions and the culture physical condition, like temperature, agitation speed, etc. , are maintained constant (99, 103) .

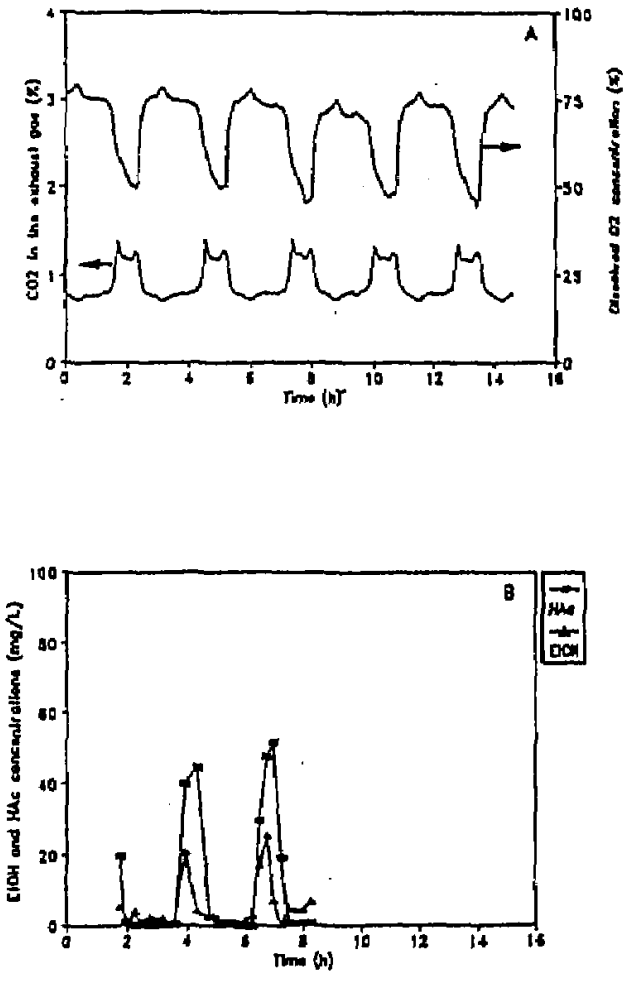


Fig. (9) Spontaneous oscillations of S. cerevisiae. [ref 104]
A. Measurements of carbon dioxide in the exhaust gas and the dissolved oxygen concentration (DOT) in % of the equilibrium value.
B. Measurements of ethanol and acetate.

This suggests that the oscillatory behavior is probably governed by microbial physiology rather these an inhomogenous environment. Nielsen and Villadsen (104) introduced an explanation to these oscillation in terms of desynchronized cell division. They gave a verbal model shown by table (1):

Table(1): **A verbal model for description of the occurrence of spontaneous oscillations in *S. Cerevisiae* cultures.**

During the single cell phase (undbudded phase) there is an accumulation of internal storage carbohydrates in the form of trehalose and glycogen. During the budding phase there is a high energy demand and the internal storage carbohydrates are mibilised, which results in a very high flux through the glycolysis. Due to the limited respiratory capacity of the cell this high flux results in excretion of ethanol. The excereted ethanol can be metabolized by the unbudded cells together with the glucose (which is present only in a limited amount) resulting in a higher specific growth rate for these cells. Furthermo: ... the presence of ethanol the critical mass for budding will become smaller while the critical mass for cell division increases. When the ethanol is exhausted the specific growth rate decreases, and the critical mass for cell division decreases whereas that for budding increases. The variations in the threshold values, i.e. the critical masses for cell division and budding, may give rise to the formation of an attractor which results in a stabilization of the partially synchronized culture.

Hjortso and Nielsen (105) based on this verbal model ; argue that periodic behavior in microbial cultures can be modeled by periodic solution of the age distribution balance. But due to the difficulties of forming a mathematical model within the age distribution framework, simulation of spontaneous oscillations has not been done by computer models which are derived from the concepts of the verbal model (104) . Since the exact mechanisms behind the occurrence of these oscillations are not fully known at present time, modeling these oscillations presents a challenging task. This task is further complicated by the need to understand the complex interactions between the different species in the process and the difficulty in quantifying the different rates involved. A computer model based on the bottleneck model (106) was proposed for instance by Strassle et al. (107) for the synchronization of Saccharomyces cervisaie cultures. Cell mass for unbudded cells and cell age for budded cells were taken as the characteristics intercellular variables for the different morphological terms. The spontaneous oscillations were correctly predicted (108) by the model. However, Strassle et al. (107) divided the biomass into large number of morphological forms (96 species), and conversion between the forms is described in a complex fashion. It is therefore difficult from their paper to extract the kinetics for metamorphosis reaction in the verbal form described by Nielsen and Villadsen (104).

Cazzador et al. (109, 110) discuss a computer model which predicts spontaneous oscillations of a budding yeast cultures. The model is also based on the desynchronized cell division mechanisms. In this model budded and unbudded cells are described as two morphological forms with the same specific growth rate given by Monod expression. The yield coefficient of biomass on glucose is, however, different for the two forms, and its hereby possible to describe oscillations in the glucose concentration when the budding index (the fraction of budded yeasts) varies. Although Yano and Koga (111) on the other

hand analyzed the behavior of continuous culture systems based on several fermentation kinetics in which the products inhibit the growth rate of the biomass. Repeated oscillations appear in system in which the inhibitory action of compound is delayed. A delayed inhibition can be imagined if the inhibitor does not act directly on the fermentation and thereby directly on its own formation, but indirectly by inhibiting another reaction, which is positively linked to the product formation. Jobses et al. (102) for instance proposed a structured model for oscillation in continuous culture of Z. mobilis at high ethanol concentrations. The oscillations were modeled by a structured mathematical model in which ethanol inhibits the maximum specific growth rate indirectly by inhibiting the synthesis of an internal growth rate determining compound.

Bifurcation analysis to Jobsis model has been given by Elnashaie et al. (112) and a wealth of different oscillations was obtained the simplicity of the morphological model of Cazzador et al., a stable limit cycle is only obtained by this model (104). In a more abstracted structured dynamical model for microbial cultures accounts for substrate and intermediate product inhibition. Ajbar and Ibrahim (113) observed a wealth of complex dynamic phenomena sustained oscillation, quasiperoidic oscillations, and chaotic oscillations. Some of these oscillations coexist with high conversion operating points.

Inspite of its simplicity and validity in bioreactors, unstructured models just begin to be used in modeling oscillatory behavior of bioferments (114). Ibrahim and Ajbar started by the analysis of one of the important instability creating mechanisms that is the substrate inhibition kinetics represented by the so common Haldane equation. They proved that this equation is useless in generating oscillation at all (114). Ibrahim and Ajbar developed a new unstructured pseudo homogenous floc model that accounts for substrate inhibition and oxygen limitation kinetics in addition to mass transfer limitation (113). The model may be summarized as follows :

$$\bar{S}_o - \bar{S} = \phi_1 \bar{\mu}_1 + \phi_2 \bar{\mu}_2 + \frac{d\bar{S}}{dt} \qquad (11)$$

$$\bar{C}_o - \bar{C} = \phi_3 \bar{\mu}_1 + \phi_4 \frac{d\bar{C}}{dt} \qquad (12)$$

The normalized rate expressions $\bar{\mu}_1$ and $\bar{\mu}_2$ have the following expressions:

$$\bar{\mu}_1 = \frac{\bar{S}}{(1+\bar{S}+\bar{S}^2/\bar{K})} \frac{\bar{C}}{(1-\bar{C})} \qquad (13)$$

$$\bar{\mu}_2 = \frac{\bar{S}}{\bar{S}+\beta} \qquad (14)$$

$$\phi_1 = \frac{\rho V_c \mu_{m1}}{Kg_s A_c K_s} \quad , \quad \phi_2 = \frac{\rho V_c \mu_{m2}}{Kg_s A_c K_s Y_{o_s}}$$

where :

$$\phi_3 = \frac{\rho V_c \mu_{m2}}{K_{g_o} A_c K_c} \quad , \quad \phi_4 = \frac{Kg_s}{Kg_c} \quad , \quad \bar{K} = K_c / K_s, \beta = \frac{K_x}{K_s}$$

In the case of fixed bulk conditions the system of equations exhibit periodic solutions for a wide range of model parameter values. The model confirms the importance of the DO levels on the existence of periodic behavior. In the case of varying the bulk conditions $[C_o^- = C_{ob}^- + A_m \sin wt]$ *the system exhibits very rich dynamic characteristics of the chaotic behavior. Birhythmicity and even trirhythmicity have been observed as shown by Fig.(10)*

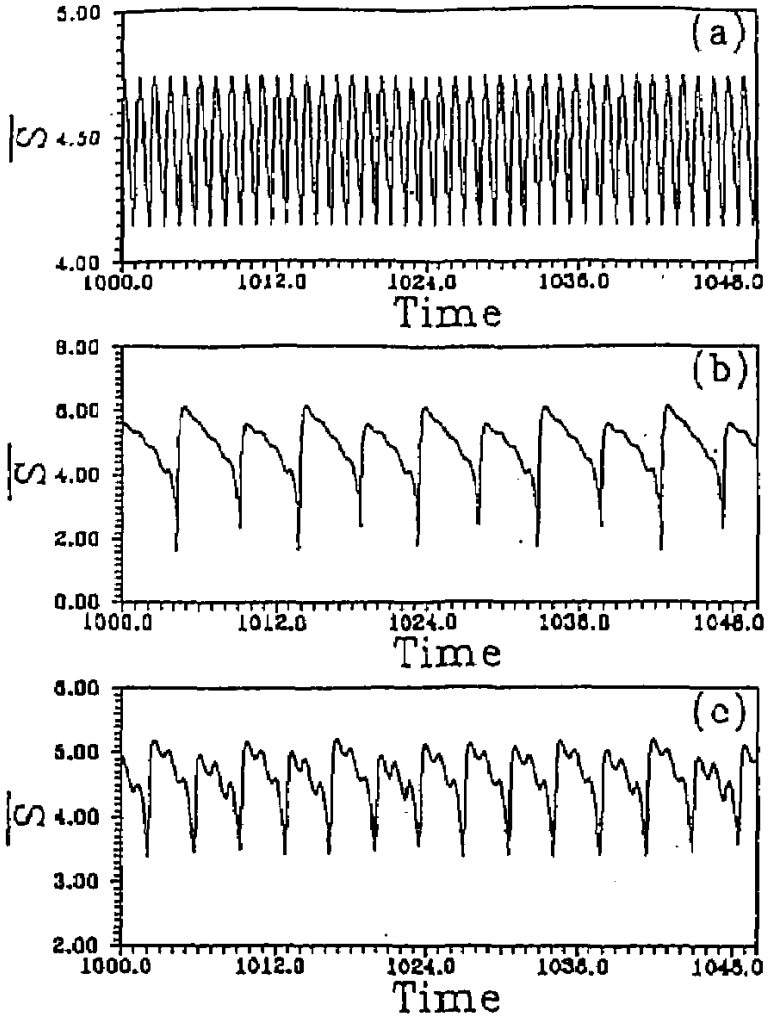


Fig. (10) Time traces showing trirhythmicity obtained by means of Eqs. 11-14.
a. Period one attractor b. Period 8 attractor ; c. Chaotic attractor [ref. 114]

I-6 Acetylcholinesterase Enzyme and Oscillating pH

Acetylcholinesterase enzyme acts on acetylcholine producing choline and acetic acid (which is fully ionized to acetate ions and H^+). This enzyme plays a recognized role in nerve excitation (116). Acetylcholine is secreted by neurons in many areas of the brain, but specifically by the large pyramidal cells of the motor cortex, and by many different neurons that innervate the skeletal muscles. A significant portion of this enzyme is found in intercellular compartments (117). In most cases, acetylcholine has excitatory effects (116).

In enzyme membrane systems, the local production of hydrogen ions decreases the pH, and owing to the amphoteric properties of the proteinaceous membrane, the lower the pH, the lower the density of the negative fixed charges in the membrane (118). Local pH changes inside an artificial enzyme membrane were first shown by Goldman et al. (119). The potential differences from acetylcholinesterase membrane exhibits similar electrical response to the behavior observed with excitable membranes (17). The steady state potential resulting from the enzyme activity for increasing and decreasing substrate concentrations exhibits a hysteresis behavior . Because of the autocatalytic effect resulting from the production of hydrogen ions and the existence of diffusional resistances, hysteresis phenomenon develops in definite range of parameters, and because of the amphoteric properties of the membrane, the hysteresis of the internal pH is transformed into a hysteresis in membrane potential (17). In addition, the nonmonotonic behavior of the enzyme reaction rate coupled with the diffusion constraints causes instabilities and oscillatory behavior in membrane potential and in acetylcholine concentration level.

The static bifurcation problems of this enzyme system has been talked by Elnashaie et al. (120-122) almost fifteen years ago. In the light of recent advances in fundamental knowledge and techniques regarding such dynamical systems, the problem is reinvestigated by Ibrahim and Elnashaie (54) on a higher level of model formulation and analysis of the results, and a wealth of new dynamic features are observed by Ibrahim et al. (54).

The problem investigated is that of the enzymatic reaction $S \rightarrow P_1 + P_2^- + H^+$

where in the case of acetylcholinesterase
S denotes acetylcholine $\{CH_3CO.O(CH_2)_2N^+(CH_3)_3\}$
P_1 denotes choline $\{HO(CH_2)_2N^+(CH_3)_3\}$
P_2 denotes acetate $\{CH_3COO^-\}$

The reaction is considered to take place in a constant flow isothermal continuous tank reactor (CSTR) which is divided by a semipermeable membrane into two compartments as shown in Fig.(11). The reaction takes place in the liquid phase according to the following rate equation (1)

$$R(S,H) = \frac{V_m[S]}{[S]+[S]^2/K_i + K_s\{K_h + [H] + \frac{2}{\lambda}/K'_h\}/[H]} \qquad (15)$$

where [S] is the substrate concentration, [H] is the hydrogen ions concentration, V_m is the maximum reaction rate per unit mass of the enzyme; K_h, K_s, K_h are equilibrium constants and K_i is an inhibition constant. If the active volumes of the compartments are V_1, V_2 and the enzyme concentrations in both compartments are equal to E in units of enzyme mass per unit of active volume, the volumetric flow rate is q. Using the pesudosteady state assumption for hydroxyl ions (i.e. [OH]/dt = 0) then the material balance equations for the two compartments can be summarized in dimensionless form as follows [for more details regarding the model derivation see reference (54).

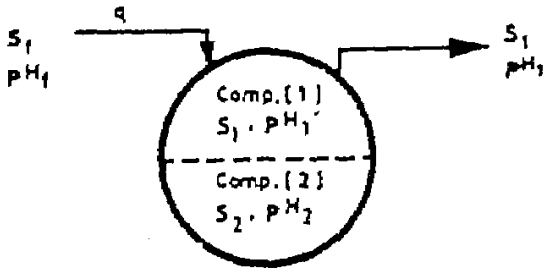


Fig. (11) The two compartments model

For hydrogen:

$$\frac{dh_j}{dT} = a_{1j}(h_f - h_1) - b_j\alpha_H(h_1 - h_2) - a_{1j}\gamma\,(1/h_f - 1/h_1) + B_h r_j + b_f\gamma\alpha_{OH}(1/h_1 - 1/h_2) \quad ..(16)$$

For substrate:

$$\frac{ds_j}{dt} = a_{1j}(s_f - s_1) - b_j\alpha_s(s_1 - s_2) - B_s r_j \qquad (17)$$

where j=1,2 denotes compartments 1 and 2 respectively, $h=[H]/K_h$, $T=t.q/V_1$, $a_{11}=1, a_{12} = 0$, f denotes feed conditions, $V_R = V_1/V_2$, $b_1 = 1$, $b_2 = -V_R$, $a_{OH} = a'_{OH}.A_m/q$, $g = K_w/k^2_h$, $B_h = V_m.E.V_1/(K_h.q)$ $a_H = a'_H.A_m/q$, $a_s = a'_s.A_m/q$, $B_s = V_m.E.V_1/(K_s.q)$, $s = [S]/K_s$, $\alpha_i = K_s/K_i$ and $\delta = K_h/K'_h$. The dimensionless rate of reaction is given by :

$$r_j = \frac{s_j}{s_j + s_j^2.\alpha_i + (1 + h_j + h_j^2.\delta)/h_j} \qquad (18)$$

The two compartments model is thus represented by four differential equations with four state variables h_1, h_2, s_1, s_2 which describe the dynamics of hydrogen ions and substrate concentrations in the two compartments.

Different dynamical models were obtained by this model including : limit cycle-bistability (point attractor and limit cycle) - chaos followed the period doubling sequence - chaos termination by crisis points - periodic windows interrupted chaotic sequence.

The transition from small amplitude oscillation to bursting oscillation proved to be quite complex (54). Fig.(12) shows an interesting feature with regard to the difference between the pH values in the two compartments (pH_1, pH_2) respectively:

$$\Delta pH = pH_2 - pH_1$$

It is clear from the figure that ΔpH changes its sign twice during each cycle (period), which means that the H^+ ions transfer between the two compartments and changes their direction twice every cycle. This alternating sign of Δ pH is associated with the bursting oscillation shown by the figure. The two modes of sustained oscillations observed in this region of parameter values may give insight into the common physiological phenomenon of slow wave rhythm of membrane potential that characterizes the self-excitation of some of the smooth muscles owing to the acetylcholinesterase activity (116). More complex dynamical modes of this system should be discussed in section II .

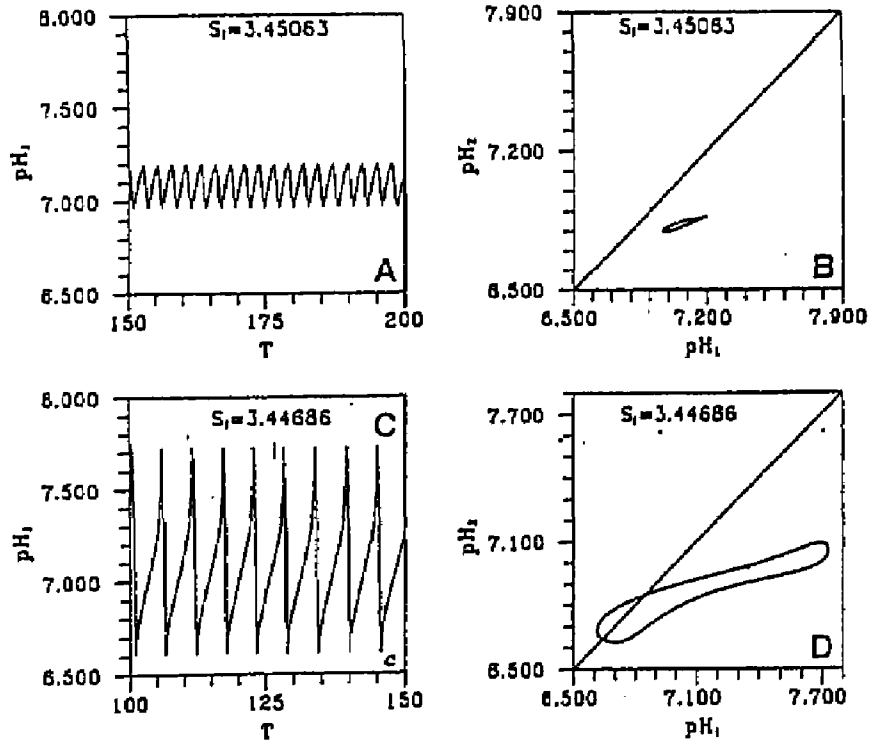


Fig. (12) Dynamic characteristics for two different values of S_1 . obtained from ref. 54 .

I-7 Periodic Behavior of Dictyostelium Cells

The cellular slime model Dictyostelium discoideum represents a prototype of spatio-temporal organization at the cellular level. These cells indeed aggregate after starvation, by a chemotactic response to pulses of cyclic -AMP (c-AMP) emitted by centers with a periodicity of 5-10 min; as a result of such a periodicity in c-AMP secretion, cells collect around the aggregation centers in a wavelike manner (120-122). From the point of view of temporal organization, the periodic synthesis of c-AMP in Dictyostelium represents a remarkable example biological rhythm involved in intercellular communication. With regard to the main theme of this volume, of particular interest is the possibility that in some cases, the signaling system in Dictyostelium may function in an aperiodic, chaotic manner. The periodic and aperiodic oscillatory properties of the c-AMP signaling system will be examined in turn below.

Model for c-AMP Signaling Based on Receptor Desensitization :The mechanism of c-AMP oscillations in Dictyostelium rests on the positive feedback exerted by extracellular c-AMP on its intracellular production. Upon

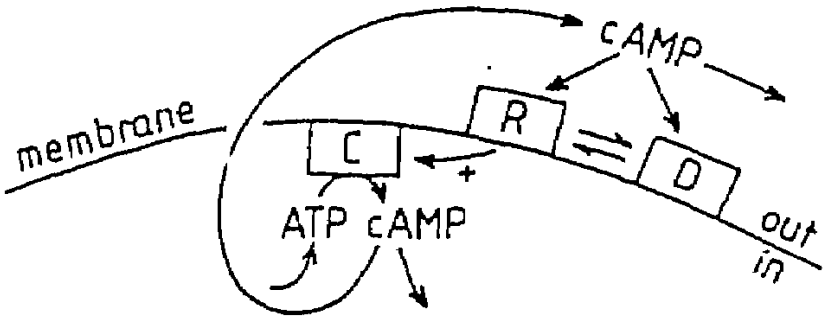


Fig.(13) Model for c-AMP signaling in Dictyostelium based on receptor desensitization . Extracellular c-AMP binds to the active ® state of the receptor and thereby elicits the activation of adenylate cyclase (C) which synthesis c-AMP from ATP. Binding of c-AMP to the receptor in the R state induces its transition to the desensitization (D) state which cannot activate the cyclase. Other arrows refer to ATP synthesis, intracellular c-AMP transport into the extracellular medium, and c-AMP hydrolysis by phosphodiesterase (from ref 123).

Binding to a cell surface receptor, c-AMP triggers the activation of adenylate cyclase which produces c-AMP from ATP. Intracellular c-AMP thus synthesized is transported into the extracellular medium where it binds to the receptor and further enhances its own production, while part of it is hydrolyzed by the enzyme phosphodiesterase present on the cell membrane and in the extracellular medium (121, 122).

As important for oscillations as is the positive feedback loop described above, the factor that limits autocatalysis is equally important. A major role here is played by desensitization of the c-AMP receptor through reversible phosphorylation (124, 125). Holding with these observations, a model for c-AMP signaling based on receptor desensitization (see Fig.13) has been proposed by Martiel and Goldbeter (6, 123). In its simplest version, it describes the time evolution of intracellular and extracellular c-AMP and of the fraction of active c-AMP receptor, ATP is treated as a parameter in that version of the model since the amplitude of ATP variations remains reduced in the course of c-AMP oscillations. The resulting three-variable system is governed by the following kinetic equations.

$$\frac{dQ_T}{dt} = -f_1(\gamma)Q_T + f_2(\gamma)(1 - Q_T)$$

$$\frac{d\beta}{dt} = q\sigma\phi(Q_T, \gamma, \alpha) \qquad (19,20)$$

$$\frac{d\gamma}{dt} = \left(\frac{k_t\beta}{h}\right) - k_e\gamma \qquad (21)$$

where,

$$f1(\gamma) = \frac{k_1 + k_2\gamma}{1+\gamma}, f_2(\gamma) = \frac{k_1L_1 + k_2L_2c\gamma}{1+c\gamma} \qquad (22)$$

$$\phi(Q_T, \gamma, \alpha) = \frac{\alpha(\lambda\theta + \epsilon Y^2)}{1+\alpha\theta+\epsilon Y^2(1+\alpha)}, Y = \frac{Q_T\gamma}{1+\gamma} \qquad (23)$$

In these equations, β and γ denote the normalized concentrations of intracellular and extracellular c-AMP while ρ_T denotes the fraction of active receptor, α is the normalized level of ATP and other parameters relate to the kinetic characteristics of the receptor, adenylate cyclase c-AMP transport, and c-AMP hydrolysis by phosphodiesterase (see ref 123 for further details).

A recent extension of the model incorporates G proteins which act as intermediates between c-AMP binding to the receptor and activation (or inhibition) of adenylate cyclase . The results obtained in that extended model are qualitatively similar to those obtained in the simpler model governed by Eqs.

*Oscillations and Relay of c-AMP Signals :*The model described by Eqs.19-23 generally admits a unique steady state solution which can become unstable for appropriate values of the parameters. In these conditions, the system evolves toward a stable limit cycle corresponding to sustained oscillations in c-AMP . These oscillations

(Fig. 14) whose period is of the order of 5-10 min for the parameter values collected from the experiments, are accompanied by a periodic alteration of the receptor between its active and desensitized states (123) . A similar alternation is observed in the experiments, since the receptor oscillates between its phosphorylated and dephosphorylated forms in the course of c-AMP oscillations (122).

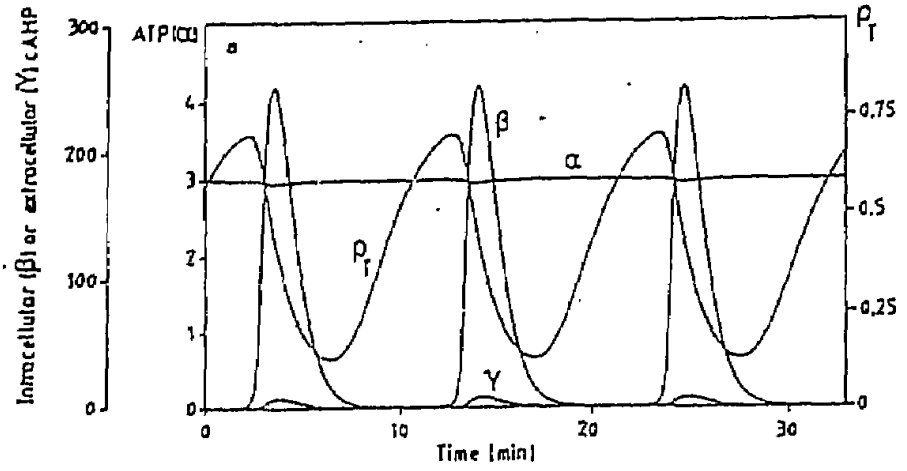


Fig.(14): Sustained oscillations of c-AMP in the model based on receptor desensitization. Shown are the normalized concentrations of intracellular (b) and extracellular c-AMP (γ), ATP (α), and the fraction of active c-AMP receptor (ρ_T) (From ref 123).

Relay of c-AMP signals in the course of aggregation consists in the pulsatory amplification of suprathreshold c-AMP pulses. The analysis of the model in the phase

plane indicates that a close link exists between relay and oscillatory behavior (123). The former type of phenomenon reflects the excitability of the c-AMP signaling system; for parameter values close to those producing oscillations, the steady state is indeed stable but excitable; a suprathreshold increase in extracellular c-AMP then leads to the synthesis of a large pulse of c-AMP before the system returns to the stable steady state.

II- Complex Oscillatory Phenomena in a Regulated Biochemical System

To analyze the transition from simple to complex oscillatory phenomena, two complementary ways exist a priori. The first is to try to account for complex oscillations observed in the experiments. The second is to rely on the analysis of more or less abstract models whose primary goal is not so much to account for experimental results but rather to explore the realm of possible modes of complex behavior . To this approach belong a series of simple biochemical models which were developed to investigate the occurrence of birhythmicity (coexistence of more than one cyclic attractors) bursting and chaos in biochemical systems (6) . The interest of these studies is of course not limited to the biochemical field because the phenomena predicted by these models can occur in other, nonbiological, chemical systems. Thus birhythmicity was demonstrated in a chemical system (51) following its theoretical prediction in one of these biochemical models (33).

II-1 Birhythmicity:

The vast majority of oscillation in biochemical system corresponds to the evolution toward a stable limit cycle. Such a limit cycle is generally unique for a given set of parameter values. A good example of the simple birhythmicity [where two unit cycles are coexist] is given by Moran and Goldbeter (52). They extended the glycolytic oscillator model to account for nonlinear recycling of product into substrate. In addition to simple periodic behavior corresponding to the evolution toward a unique limit cycle the system can admit the coexistence between two stable limit cycles more recently Ibrahim and Elnashaie (53) discovered a complex birhythmicity in the enzyme acetylcholinesterase system using two compartments model (54) . This complex bistability are summarized as follows.

* Two simple periodic attractor (two cycles of counted periodicity)
* Complex bursting and simple periodic attractor .
* Chaotic and simple periodic attractors.
* Complex bursting and chaotic attractors .

These four categories are summarized and shown in Fig.(15).

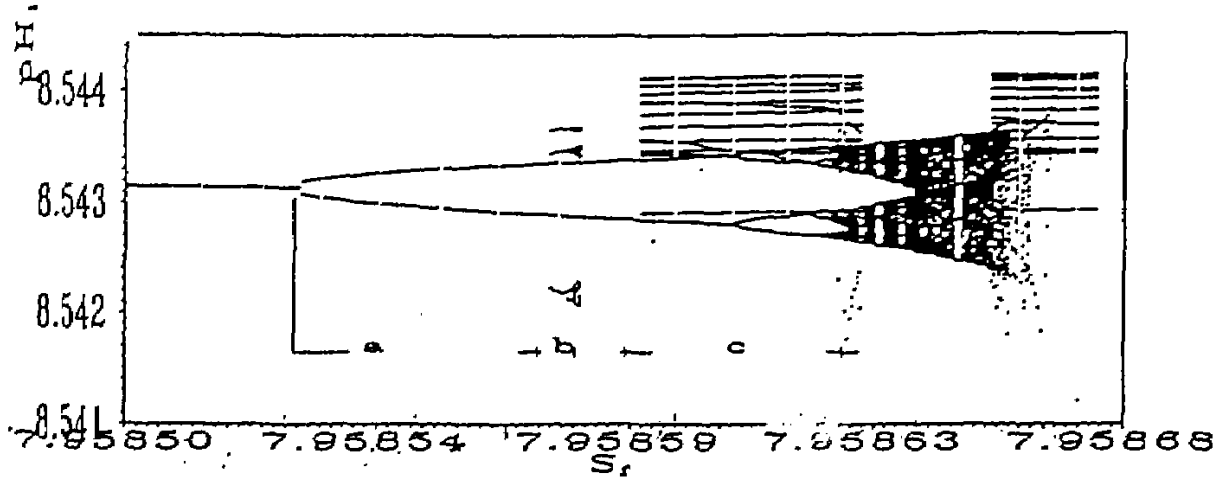


Fig. (15) Complex bistability obtained by means of the two compartments model schematized by Fig. 11. [ref. 53]

Actually this complex birhythmicity obtained in the negbourhood of dangerous boundaries, i.e. homoclinical condition.

Ibrahim and Ajbar found sense few months a very rich bistability when they developed and analyzed a *pseudo-homogenous floc model for oxygen limited* fermentation processes (55). They found a region of parameter values characterized by three cyclic attractors one of these attractors was chaotic and the second was high periodic and the last was period one attractor as shown- elsewhere- by Fig.(10). However, the phenomena of birhythmicity and trirhythmicity between all the attractors coexisting in phase space (two or three) indicates dynamical richness and could be controlled upon appropriate perturbations.

II-2 Bursting and Chaos:

Bursting is a term used to describe the behavior of certain neurophysiologycal and chemical systems in which there is a period of rapid spiking followed by quiescent (resting) period. It is often in this form of excitable membrane activity that cells in the biological nervous systems are involved in various rhythmic behavior, such as central pattern generations in invertebrates or pacemakers of brain waves in mammalian cortex

(56, 8) . It is therefor a question of biological interest to ask how a rhythm (of bursting) can come about (57). The work of Holden and Fan (58-61) on the dynamic behavior of membrane excitation using a non-phenomenological three variables model of action potential shows clearly the existence of different dynamic modes, including simple periodic, bursting periodic and chaotic behavior. A wealth of transition mechanism between different types of behavior has been discovered by Holden and Fan (58-61).

One of the interesting observations noticed is that many of the dynamic phenomena discovered by Holden and Fan using the three dimensional non-phenomenological action potential model (Rose-Hindmarsh Model) are also obtained using the present phenomenological two compartments model with membrane separating the two compartments (54).

Ibrahim and Elnashaie observed and analyzed the bursting generation in the acetylcholineserase system via complex bifurcation scenario. This complex scenario occurs in the neighborhood of homoclinic orbits. They proved the homoclinicity condition using the generalized criterion which was developed by Rossler et al. (62) to extend Silinikov theorem of homoclinicity to the case of four dimension system [The two compartments model used by Ibrahim and Elnashaie is four dimensional model]. Ibrahim and Elnashaie on trying to answer the question of how these modes complex bursting come from . They recognized homoclinical conditions associating these complex modes of bursting generation (53).

A simple example of this type of oscillatory behavior is obtained from a three variable model which has been developed by Goldbeter and Decroly (33, 63) . This model is based on the allosteric model proposed for glycolytic oscillation (1). Here, two allosteric enzyme are coupled in series, each of these enzyme is activated by its reaction product Fig.(16). The variety of dynamic behavior obtained by this model is much larger than in the system comprising a single positive feedback loop . Here, one can observe the following mode of oscillations :

1) Simple periodic oscillation (limit cycle). 2) Hard excitation (i.e. coexistence of a stable limit cycle with a stable steady state).3)Chaos , which is reached after a sequence of period doubling bifurcation . 4) Bursting oscillation. Multiple periodic regions also can be obtained in this model up to three coexisting stable limit cycles (trirhythmicity) have thus been found. Similar modes of dynamic behavior have been observed in another biochemical model containing an autocatalytic step (64).

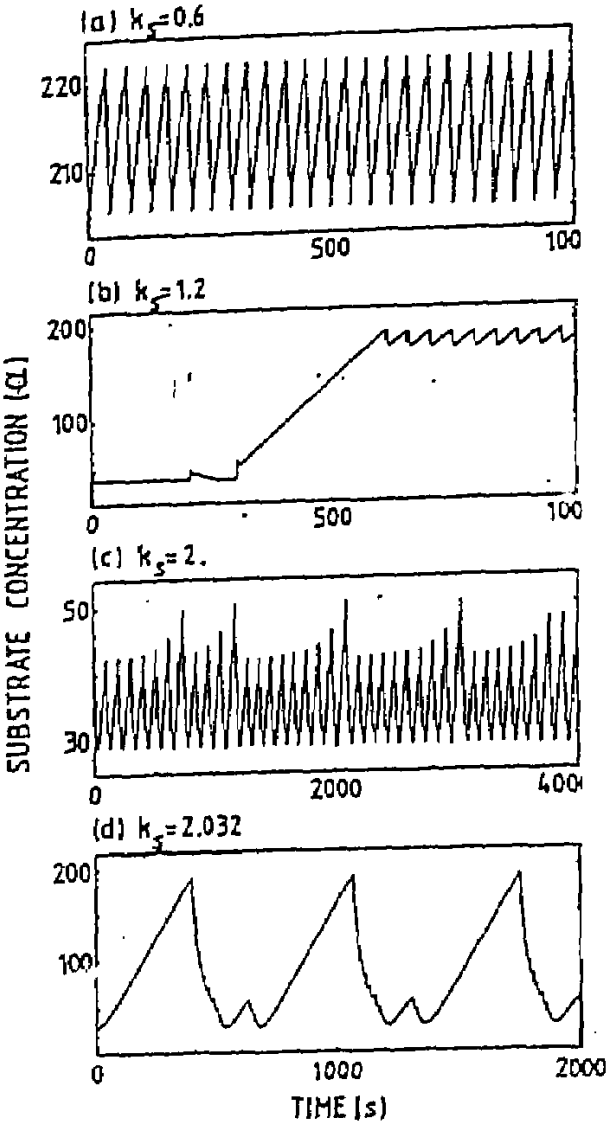


Fig. (16) From simple to complex oscillatory phenomena in the multiply regulated biochemical system [ref . 1]

To explain the occurrence of such complex dynamic modes, Goldbeter (1) urged that" Bursting and chaos clearly originate in this multiply regulated biochemical system from the interplay between two instability-generation mechanisms. Each of the two positive feedback loops can indeed produce on its own sustained oscillation for appropriate parameter values. Complex oscillatory phenomena result from the interaction of the two instability mechanisms when these become active at the same time".

An empirical method for finding domains of complex oscillations and chaos in parameter space has been developed based on this conjecture (33). It consists of identifying two distinct domains of instability in a given parameter space, each of which is associated with one of the two destabilizing feedback mechanisms. Then, by changing some other parameter, the two domains of instability are brought closer to each other until they overlap. It is often in the region of overlap that complex oscillatory phenomena occur in the form of birhythmicity, bursting or chaos.

Investigation of the Martiel and Goldbeter (123) model of the Dictyostelium cells oscillation for more complex dynamical modes (i.e. Bursting and Chaos) are detailed in reference (6). However, taking into account the variation of ATP, the signaling system is governed by a set of four kinetic equations. A quasi-steady state hypothesis for intracellular c-AMP (β) permits one, however to reduce the number of variables so that the system is governed by the following set of three kinetic equations (126).

$$\frac{dQ_T}{dt} = -f_1(\gamma)Q_T + f_2(\gamma)(1 - Q_T)$$

$$\frac{d\alpha}{dt} = \nu - \sigma\phi(Q_T, \gamma, \alpha)$$

$$\frac{d\gamma}{dt} = \frac{qk_t\sigma\phi(Q_T, \gamma, \alpha)}{h(k_t + k_t)} - k_e\gamma \qquad (23,24,25)$$

Where:

$$f_1(\gamma) = \frac{k_1 + k_2\gamma^2}{1+\gamma^2}, f_2(\gamma) = \frac{k_1L_1 + k_2L_2c^2\gamma^2}{1+c^2\gamma^2} \qquad (26)$$

$$\phi(Q_T, \gamma, \alpha) = \frac{\alpha(\lambda\theta + \epsilon\gamma)}{1+\alpha\theta+\epsilon Y(1+\alpha)}, Y = \frac{Q_T\gamma^2}{1+\gamma^2} \qquad (27)$$

Besides the inclusion of α as a variable and the quasi-steady state assumption for β, what distinguishes Eqs.23-27. is the hypothesis that the nonlinearity required for oscillations occurs in the activation step between the receptor and the cyclase rather than at the level of c-AMP binding to the receptor. Both kinds of nonlinearity yield similar types of dynamic behavior.

The system governed by Eqs.23-27 presents new modes of oscillatory dynamics, in addition to simple periodic behavior and relay of suprathreshold c-AMP signals. Thus bursting and birhythmicity can occur in this system, (126) as well as aperiodic oscillations (127), the latter again appear through a cascade of period-doubling bifurcations, both with respect to parameter ν and to parameter k_e which relate, respectively, to the input of ATP and to the hydrolysis of extracellular c-AMP by phosphodiesterase. The domains of birhythmicity and chaos in parameter space are again much more reduced than those where simple periodic oscillations or bursting are found (6).

The prediction of chaotic behavior in the model for c-AMP signaling raises the possibility that autonomous chaos may occur in the course of D. *Discoideum* aggregation (127). Of particular interest is the observation by Durston (128) that in contrast to wild type amoebae which aggregate in a periodic manner on agar after starvation, aggregation is "aperiodic" in the mutant Fr17; whereas the interval between successive waves remains close to 5 min in the wild type, it varies from 4 to 24 min in Fr17. Preliminary evidence from experiments in cell suspensions of the mutant HH201 derived from Fr17 indicates that the synthesis of c-AMP in that mutant is "erratic" (129). To test the occurrence of chaos in HH201, cells placed in stirred suspensions were studied by light scattering so as to monitor the dynamic behavior in a continuous manner (130). Instead of aperiodic behavior , rather regular oscillations were recorded, with a progressive drift in the period from 8 to 6 min, as observed in suspensions of wild type cells.

II-3 Strange Chaotic Attractors

Few months ago, Ibrahim and Elnashaie investigated the structure and strangeness of some chaotic and periodic attractors (bursting) which occur in the neighborhood of homoclinical orbits . One of these attractors is small amplitude high frequency chaotic attractor . This attractor was found to be screw type attractor according to Rossler notations. This type of attractors are a generic path to homoclinicity (65). Another type of the strange attractors which was observed by Ibrahim and Elnashaie is the mixed mode chaotic attractor.

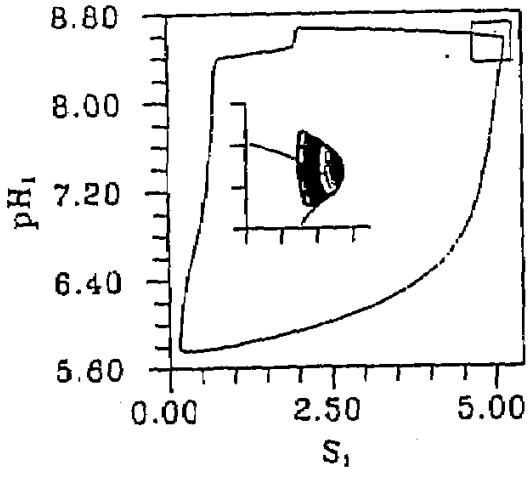


Fig. (17) Strange chaotic attractor observed by Ibrahim and Elnashaie (53)

This attractor is a composite state of two type of oscillation : complex periodic bursting attractor and chaotic attractor. Fig.(17) shows one of the strange attractors found by Ibrahim and Elnashaie. This attractor appears to be topologically strange from the following points of views: Microscopic changes in phase space. Interruption of the spiraling out sequence. The mixed mode attractor has many similar characteristics with the interesting Birkhoff-Shaw attractor (66) which is produced from the forced Van der Pol's equation, specially with regard to the feature of repetitive periodic cycles. In the case of Ibrahim and Elnashaie attractor, the repeated cycle is mainly bursting oscillation and in Birkhoff-Shaw attractor, the repeated cycle is the forced cycle. The mixed mode attractors are very important from practical point of view where the phenomenon of irregular action potential may find an explanation.

III- Controlling Chaos

In Biosystems positive as well as negative regulatory feedback provide sources of nonlinearity which, in conjunction with co-operative processes, give rise to instabilities associated with oscillatory behavior (1). Beside periodic behavior , other, more complex oscillations have been identified and increasingly studied in recent years (1). Among these complex oscillations the most commonly encountered are bursting oscillations and aperiodic oscillation (chaos). In many practical situations it is interesting to enhance the appearance of chaos in order to favor process performance such as mixing of fluids or achieving high rate of heat transfer in some process industries (132) . However, in other situations chaos may be undesirable, such as mechanical systems where chaos causes fatigue failure and also temperature oscillations outside safe regions in thermal systems.

In physiological applications, several authors (132 - 133) have discussed the question of whether chaotic behavior constitutes evidence for pathological behavior of the system or whether it indicates healthy variability universally found in living organisms and the whole of nature in general. There has been increasing evidence to support the case that chaos plays a positive role in the physiology of the organism Goldberger and co-workers (132, 136) stated that chaos is a healthy phenomenon because it provides the organism with an "information-rich (broadband) state" and "spectral reserve". However one of the fundamental aspects of chaos is that many different possible behavior are simultaneously present in the system dynamics. A particular manifestation of this is the fact that there are typically an infinite number of unstable periodic orbits that co-exist with the chaotic dynamics (137, 138).

The presence of chaos may be a great advantage for control in a variety of situations. When using small or large controls for non-chaotic systems, we are usually stuck with whatever system performance already exists. In a chaotic system on the other hand, we are free to choose between a large variety of dynamical behavior. The interested in the investigation of the problem of chaos using feedback control methods has started recently (139-141) . These methods needs the knowledge of the return map of the system and works by perturbing the system state in such a way that it leads to the

desired fixed state. Beside the feedback methods. There is a possibility to stabilize periodic orbits by nonfeedback methods (142, 143) . This interesting technique presents the advantage of being much easier to implement. It is consists of the application of resonant periodic parametric perturbations, that effectively stabilize some unstable periodic orbits of the system . The effects associated with these perturbations are generally difficult to predict nevertheless it is easy to implement . From another point of view this technique is a common way - naturally occurring way- to regulate some physiological processes of great interest in living organisms. For example the secretion of hormones (like acetylecholine) is a periodic process (116).

To investigate the effects of small periodic perturbation on chaotic behavior, Li et al. (144), tested the dynamic behavior of Dictryostelium cells comprising variable proportions of cells from two populations, one chaotic and the other periodic. For each population cells synthesize and secrete c-AMP according to Eqs 23-27 with one equation for the fraction of active c-AMP receptor (ρ_T) and one for the substrate ATP (α), but the kinetic equation for extracellular c-AMP (γ) takes into account the production of c-AMP by the two populations present within the mixed suspension, as well as the degradation of c-AMP by phosphodiesterase produced by the two types of cells.

Starting with a homogeneous population of periodic cells ($F_1 = 1$) and increasing progressively the proportion F_2 of (initially) chaotic cells, a sequence of period-doubling bifurcations leading to chaos is found (145) as a function of F_2. The physiologically most significant result, however , is that the presence of a tiny proportion of periodic cells, e.g. of the order of a few %, suffices to suppress the chaotic behavior of the large majority of cells present in the suspension (144, 145). Thus, Fig. (18) indicates that the presence of 5% periodic cells suffices to transform the (initially) chaotic behavior of 95% of the cells into complex periodic oscillations. A detailed bifurcation analysis of the mixed system shows (6) that such a sensitivity of chaos can be related to the relative smallness of the chaotic domain as compared to the domain of periodic behavior in parameter space. Mixing the chaotic and periodic cell populations amounts to shifting the effective value of control parameter in a homogeneous population out of the range of chaos into the range of values corresponding to simple or complex periodic oscillations (6).

To further check the effect of mixing a small proportion of periodic cells with chaotic ones, a population of chaotic amoebae was subjected to a small-amplitude periodic forcing (6, 145). This was done in the model by adding a sinusoidal input term to the kinetic equation for γ in Eq. 25 Confirming the results of the mixing of periodic and chaotic cells, simulations indicate (Fig. 18) that a tiny periodic perturbation of the strange attractor is sufficient to transform chaotic into periodic behavior.

Controlling chaos by small perturbation has also been studied by Ibrahim (131) using the two compartments model described before. Ibrahim examined two types of perturbing functions : sinusoidal and square function. This investigation has shown that

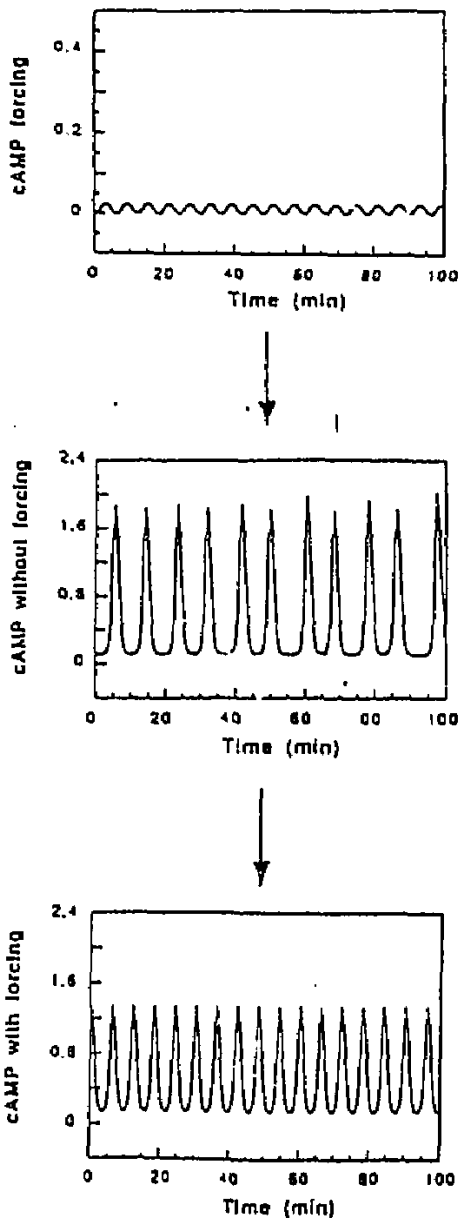
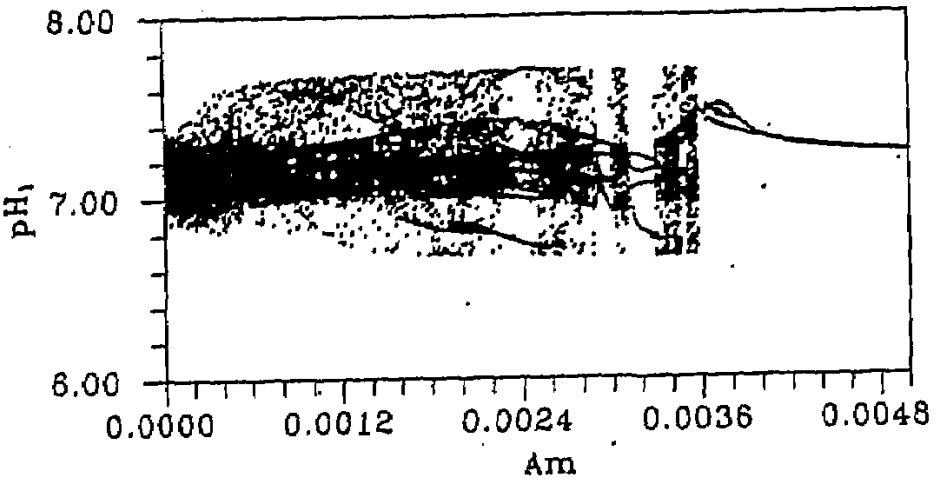


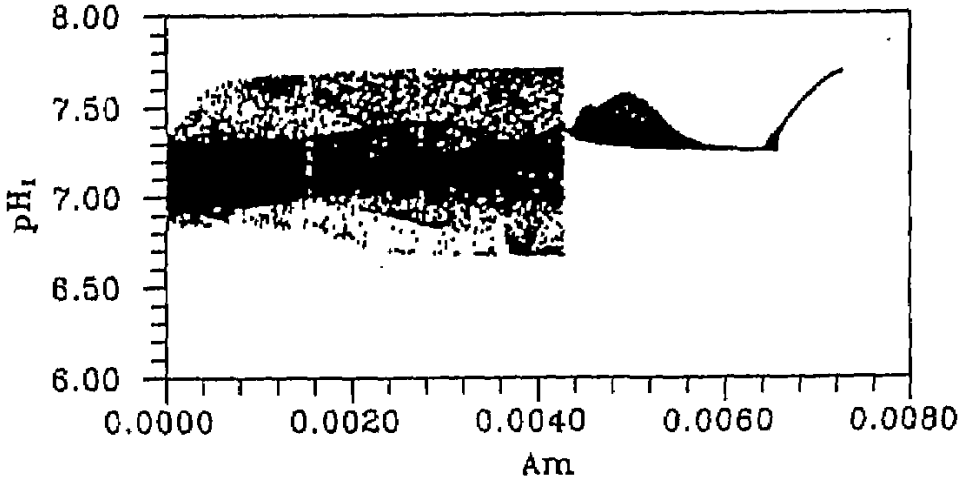
Fig. (18) Suppression of chaos by a small amplitude periodic forcing in the model for c-AMP signaling [ref. 144]

regular motion (periodic) could be obtained using small perturbation regardless of the shape of perturbed regular function. In the case of excitation with fixed frequency, full entrainment occurs at certain forcing amplitude as shown by Fig. (19). Full

entrainment of the system requires less amplitude in the case of square forcing than in the case of sinusoidal forcing. Ibrahim (131) has also shown that a regular regions interrupted by strips of chaos can emerge from the original unperturbed system in the case of excitation using fixed amplitude (changing frequency). Wider periodic windows [wider regular regions] was observed with increasing the forcing frequency.



The excitation diagram at fixed frequency using square forcing



The excitation diagram at fixed frequency using sinusoidal forcing

Fig. (19) Effects of two different forcing functions on a chaotic behavior obtained by the two compartments model [ref. 131]

Concluding Remarks

The study of biochemical oscillations has undergone great advances in the last decade An overview of the important examples of biochemical oscillators are given : PO reaction- Glycolytic reaction. - Intracellular Ca++ Oscillations - Mitotic Oscillator Acetyecholinesterase reactions - Some fermentation processes and the oscillations in Dictyostelium cells. The regular and irregular rhythms were briefly presented together with some dynamic models proposed for qualitative study of these systems.

The experimental studies of PO reaction (The first example of chaos in biochemical systems) lag , by far, the theoretical work (6) and computational studies. Some of the remaining questions involving this reaction focus on the details of the mechanism involved with the generation of regular and chaotic oscillations. Recent theoretical studies of possible detailed mechanisms bring us closer to answering these questions (36) However , these answers can be only conjectures without further experimental work. Other unsolved problems involve the role of the critical additives methylene blue and dichlorophenol. At dynamical level the origin of the oscillations and the route to chaos have only begun to be studied. Further extensive investigation of this system is necessary to approach the maximum possible understanding of the nonlinear dynamics of the PO system . Oscillations of the most industrial important microorganisms are also reviewed : S. cerviciae and Z. mobilis, one of the important observation is that, the operation of the bioreactor under chaotic motion gives higher average substrate conversion, higher product yield and higher production rates than for steady state conditions with the same bifurcation parameter (112) . Further understanding of this system (Z. mobilis) needs extensive work in the level of modeling and experimental works: the direction of modeling development may be directed to more conceptual and little empirical models. Experiments should be devoted to the operation of the fermenter at chaotic state to examine the theoretical prediction of Elnashaie et al. (112) of higher yield and higher conversion rates associated with the chaotic state.

With respect to S. cerviciae, the models based on age distribution balance (105) still far from complete representation of this important yeast culture oscillations . Actually these type of models do not permit mass transfer limitations to be treated separately within the model context. This is in contradiction with the fact that mass transfer may play a remarkable role in such systems (114) specially o_2 transfer limitations .

On the other hand the simple unstructured model given by Ibrahim and Ajbar (114) confirmed well with the experimentally observed dissolved oxygen oscillations of such a system. This model needed to extend to a macroscopic scale (bioreactor) rather than the floc scale.

The experimental autonomous oscillations of pH in the acetylcholinesterase-acetylcholine system(17), received little attention despite of its importance as a main neurotransmitter. The earlier work of Elnashaie et al. (120a,b,c) proved that this system is very rich in static bifurcation phenomena (multiplicity of steady states: more than 25 steady state were discovered - Patterns formation). The recent work of Ibrahim and Elnashaie proved that this system could be used as and important example for different type of simple and complex modes of oscillations and strange periodic and

chaotic behavior. An extensive attention should be paid to prove experimentally that dynamical richness of the system.

The function of glycolytic oscillations remains unclear, although it has been suggested that they may enhance the thermodynamic efficiency of glycolysis (146), or the ATP/ADP ratio (68) in the ATP- producing pathway. These oscillations could well be merely an accidental consequence of peculiar regulation of phosphofructokinase ; a similar view may also hold for the oscillatory peroxidase reaction. Even so, the virtue of this systems would be to provide highly useful models for biochemical oscillations that can be studied in vitro.

The existence of an optimal pattern of pulsatile stimulation by c-AMP signals in dictyostelium can be related to the optimal frequency observed in the action of a number of hormones (147-149) whose secretion also follows a pulsatile pattern. It is noteworthy that in the model based on receptor desensitization which applies both to c-AMP and hormonal signaling , the optimal periodic signal proves more effective than random or chaotic pulstile stimuli(150).

The physiological function of biochemical oscillations has been clarified to various degrees . The clearest and most important case is that of the cell division cycle which plays a major role in development. Moreover, perturbations of the continuous biochemical oscillator controlling mitosis may lead to cell cycle arrest or may conversely be involved in abnormal cell proliferation. As to Ca^{2+} oscillations, their effects only begin to be investigated; most of the work so far has indeed been devoted to the characterization of the oscillatory phenomenon. It appears that Ca^{2+} oscillations could be encoded in terms of their frequency(18-22,73,80) ; the latter indeed rises with the level of stimulation by hormones or neurotransmitters. Oscillations in cytosolic Ca^{2+} are often accompanied by Ca^{2+} waves propagating within the cell from the site of stimulation(74-77), these waves could serve in intracellular signaling as well as in propagating signals from cell to cell.

At the cellular level, the "aperiodic signaling" properties observed (128)during aggregation on agar in the Dictyostelium mutant Fr 17 could provide an example of autonomous, biochemical chaos. Attempts at characterizing in terms of chaos the behavior of this mutant in cell suspensions have failed, however, since rather regular oscillations instead of aperiodic behavior were observed(130). Because this result could well be due to the suppression of chaos by the strong coupling with some periodic cells in the mixed suspension(145,146), further studies of the chaotic mutant of Dictyostelm should best be carried out during aggregation on agar, as originally considered by Durston(128) . Any center behaving in a chaotic manner would then be capable of expressing its aperiodic nature in the absence of the strong coupling that takes place in cell suspensions.

The analysis of models for biochemical oscillations throws light on the molecular mechanisms of periodic behavior and clarifies the conditions in which simple periodic oscillations give way to more complex oscillatory phenomena, including chaos . With regard to periodic behavior, although end product inhibition can in principle give rise to limit cycle oscillations(151,152), the most prevalent type of regulation involved in generating the phenomenon is positive feedback(12). Such is the case of the phosphofuctokinase reaction and for c- AMP signaling in Dictyostelium. The regulatory

mechanisms which underlie Ca^{2+} oscillations also appear to include positive feedback in the form of Ca^{2+} -induced Ca^{2+} release . As to the mitotic oscillator, some models are again based on positive feedback, via the (not yet fully characterized) autocatalytic regulation of cdc2 kinase(90-92), but other models(6,93) show that oscillation may result solely from the delayed negative feedback present in the interactions between cyclin and cdc2 kinase. Required in all these oscillatory mechanisms is a minimum degree of nonlinearity.

A recurrent finding in all models investigated for complex oscillations is that however complex the regulatory structure of the biochemical system may be, simple periodic oscillations by far remain the most common mode of dynamic behavior, followed by complex periodic oscillations in the form of bursting. The occurrence of multiple stable limit cycles and chaos is restricted to much smaller domains in parameter space. This may explain the fact that for most oscillations observed in biological systems, at least in autonomous conditions periodic behavior prevails over chaos.

References

1- Richard , J.F. and Laszlo,G. , Chaos in Chemistry and Biochemistry, Richard J Field and Laszlo Gyorgyi, eds. World Scientific Publishing Co. Pte. Ltd. (1993)
2- Lorenz,E.N. , J. Atmos. Sci, 20 , P 130 (1963)
3- Elbert, T. , Ray, W.J., Kowalik, Z.J., Skinner, J.E , Graf, K. E and Biraumer, N. , Physiole. Rev., 74,no1, P1 (1994).
4- Skarda, C.A. and W.J. Freemen , Behav. Brain Sci, 10, P 161 (1987)
5- Winfree , A.T. , " The Geometry of Biological Time", Springer, New York (1980)
6- Goldeter, A. "Biochemical Oscillatioons and Cellular Rhythms" Cambridge University Press, ISBN 0521403073 (1996).
7- Olsen, L.F. and Degn, H. , Quart. Rev. Biophys. , 18, P 165 (1985).
8- Glass, L. and Mackey, M.C. "From Clocks to Chaos: The Rhythms of Life" Princton Univ. Press, Princeton , NJ, (1988).
9- Gerisch, G. and Wick, U., Biochem. Biophys. Res. Commun., 65, P 364 (1975) .
10- Chance, B., Hess, B. and Betz, A. , Biochem Biophys. Commun. , 16, P 182(1964)
11- Pye, E.K. , Can. J. Bot., 47, P 271 (1969)
12- Hess, B. and Boiteux, A. , Annu. Rev. Biochem., 40, P237 (1971)
13- Berridge, M.J. and Rapp, P.E. , J. Exp. Biol., 81, P 217 (1979).
14- Goldbeter, A. and Gaplan, S.R. , Annu. Rev. Biophys. Bioeng., 5, P 449 (1976).
15- Yamazaki, I., Yokota ,K. and Makajima, K., Biochem. Biophys. Res. commun. , 21,P 582 (1965)
16- Chance, B. and Yoshioka, T. , Arch. Biochem. Biophys. , 117, P 451 (166)
17- Friboulet, A., David, A., and Tomas, D. , J. of Membrane Sci., 8, P 33 (1981)
18- Guthberston, K.S.R. and Cobbold, P.H. , Nature, 316, P 451 (1985) .
19- Berridge, M.J. and Galione, A., FASEB J, 2, P 3074 (1988) .
20- Tsien, R.W. and Tsien, R.Y. , Annu Rev. of Cell Biol. , 6, P 715 (1990).
21- Meyer, T. and Stryer, L., Annu. Rev. Biophysics Biophys. Chem., 20, P 153 (1991).
22- Cuthbertson, K.S.R. and Cobbold, Eds, "Cell Calcium", 12,P 2 (1991).
23- Murray, A.W. and Kirschner, M.W. , Science, 246, P 614 (1989).
24- Degn, H. , Nature, 217, P 1047 (1968).
25- Degn, H., Biochem. Biophys. Acta, 180, P 271 (1969).
26- Nakamura, S. , KoKota , K. and Yamazaki, I. , Nature, 222, P 794 (1969).
27- Olsen, L.F. and Degn, H., Biochim. Biophys. Acta, 523, P 321 (1978)
28- Olsen L.F. and Degn, H. , Nature, 267, P 177 (1977).
29- Li, T. Y. and Yorke , J. , Am . Math. Mon., 82, P 985 (1975).
30- Rossler, O.E. , Naturforsch ,31A, P 259 (1976).
31- Schmitz, R. A. , Graziani , K.R. and Hudson, J.L. , J. Chem. Phys. , 67, P 3040 (1977).
32- Markus, M., Kuschmitz, D. and Hess, B. , FEBS Lett, 172, P 235 (1984).
33- Decroly, O. and Goldbeter, A. , Proc. Natl Acad. Sci USA, 79, P 6917 (1982).
34- Geest, T. , Steinmets, G.G. , Larter, R. and Olsen, L.G. , J. Phys. Chem., 96, P 5678 (1992).
35- Aguda, B.P. and larter, R. , J. Am. Chem. Soc., 113, P 7913 (1991).
36- Samples, M. , Ph. D. thesis, Stanford Univ. (1992).

37- Degn, H., Olsen, L.F. and Perram, J.W. , Ann. N.Y. Acad. Sci. , 316, P 623 (1979).
38- Larter, R., Bush, C.L., Lonis, T.R. and Aguda, B.D., J. Chem. Phys. , 87, P 5755 (1987) .
39- Larter, R. , Steinmetz. C.G. and Aguda, B.D. , J. Chem . phys. , 89, P 6506 (1988).
40- Olsen, L. F. , Phys. Lett. , 94A, P 454 (1983)
41- Yokota, K. and Yamazaki, I. , Biochem., 16, P 1913 (1977).
42- Fed'kina, V. , Ataulakhanov, F. and Brannikova, T. , Biophys. Chem., 19, P 259 (1984).
43- Aguda, B.D. and Clarke, B.L., J. Chem. Phys. , 87, P 3461(1987)
44- Aguda, B.D. and Larter, R., J.Am.Chem. Sci. , 112, P 2167 (1990).
45- Aguda, B.D., Hofmann Frisch L.L. and Olsen, L.F., J. Am. Chem. Soc. , 112, P 6652 (1990).
46- Lazar, J.G. and Ross, J., Science, 247, P189 (1990).
47- Lazar, J.G. and Ross, J.,J. Chem. Phys., 92, P 3579 (1990) .
48- Halliwel, B., Planta, 140, P 81 (1978).
49- Sevcik, P. and Dunford, H.B., J. Phys. Chem., 95, P 2411 (1991).
50- Burger, M. and Field, R.J., Nature, 307, P 720 (1984).
51- Alamgir, M. and Epstein, I.R., J. Am. Chem . Soc., 105, P 2500 (1983).
52- Moran, F.and Goldbeter, A., Biophys. Chem., 20. , P149 (1984).
53- Ibrahim, G. and Elnashaie, S.S.E.H, Chaos Solitons and Fractals, Prgamon Press, No 704.
54- Ibrahim, G. , Elnashaie, S.S.E.H, and Teymour, F. , App. Biochem. & Biotech, 55, P 175 (1995).
55- Ibrahim, G. and Ajbar, A. , TESCE , (July 1997) .
56- Doyon, B., Acta-Biotheor. , 40, P 113 (1992) .
57- Wang, X.J., Physica D, 62, P263 (1993)
58- Holden, A.V. and Fan, Y.S., Chaos Solitons and Fractals, 2, No3, P221 (1992).
59- Holden, A.V. and Fan , Y.S., Chaos, Solitons and Fractals, 2 No4, P 349 (1992).
60- Holden, A.V. and Fan, Y.S. , Chaos, Solitons and Fractals, 2 No 6, P 583 (1992).
61- Fan, Y.S. and Holden, A.V., Chaos, Solitons and Fractals, 3no4, P 439 (1993).
62- Rossler, O.E., Hudusn, J.L. and Rossler, R., Physica D., 62, P 80 (1993).
63- Decroly, O. and Goldbeter, A, J. of Theor. Biol. , 124, P 219 (1987) .
64- Tracqui, P., Perault, A.M. Milhaud, G. and Staub, J.F., Bull. Math. Biol., 49, P 597 (1987).
65- Arneodo, A., Argoul, F., Elezgaray,J. and Richetti, P., Physica D, 62, P134 (1993) .
66- Hess, B. Boiteux, A. and Kruger, J. Adv. Enzyme Regul. , 7, P 149 (1969) .
67- Frenkel, R. , Arch. Biochem. Biophys., 125 , P 151 (1968).
68- Tornheim , K, Andres, V. and Schultz, V., J. Biol. Chem., 266, P15675 (1991).
69- Higgins, J. , Proc. Natl. Acad. Sci, USA , 51, P 989 (1968) .
70- Sel'kov, E.E., Eur. J. Biochem., 4, P 79 (1968).
71- Goldbeter, A. and Lefever, R., Biophys. J., 12. P 1302 (1972) .
72- Monod, J., Wyman, J. and Changeux, J.P., J. Mol. Biol., 12, P 88 (1965).
73- Berridge, M.J., Cobbold, P.H. and Cuthbertson, K.S.R., Phil. Trans. R. Soc. Lond. B320, P 325 (1988).

74- Gilkey, J.C. Jaffe, L.F., Ridgway, E.B. and Reynolds, G.T. , J. Cell biol., 76, P 448 (1978).
75- Rooney, T.A., Sass, E.J. and Thomas, A.P., J. Biol Chem., 265, P 10792 (1990).
76- Jacob, R., Cell Calcium, 11, P 241 (1990)
77- Takamatsu, T. and Wier, W.G. , FASEB J., 4, P 1519 (1990).
78- Dupont, G. and Goldbeter, BioEssays, 14, P 485 (1992) .
79- Meyer, T.and Stryer, L., Proc. Natl. Sci. USA, 85, P 5051 (1988)
80- Goldbeter, A. , Dupont, G. and Berridge, M.J., Proc. Natl. Acad. Sci USA, 87, P 1461 (1990).
81- Dupot, G., Berridge, M.J. and Goldbeter, A., Cell Calcuim, 12, P 73 (1991).
82- Finch, E.A., Turner, T.J. and Godin, S.M., Science, 252, P 443 (1991).
83- Dupont, G. and Goldbeter, A., Cell Calcuim,(1992).
84- Girard, S. , Luckhoff, J., Lechleiter, J., Sneyd, J. and Clapham, D., Biophys. J. , 61, P 509 (1992).
85- Nurse, P., Nature, 344, P 503 (1990).
86- Murray, A.W. and Kirschner, M.W., Nature, 339, P 275 (1989)
87- Draetta, G., Trends Biochem. Sci, 15, P 378 (1990) .
88- Lora, T. , Fesquet, D. , Zindy, F. , Le Bouffant, F. , Cerruti, M. , Brechot, C., Devauchelle, G. and Doree, M., Mol. Cell Biol.,11, P1171 (1991).
89- Karsenti, E., Verde, F. and Fedix, M.A., Adv. Protein Phosphatases, 6, P 453 (1991).
90- Hyver, C. and Le Guyader, H., Biosystems, 24 , P 85 (1990).
91- Norel, R. and Agur, Z. , Science, 251, P 1076 (1991).
92- Jyson, J.J., Proc. Natl Acad. Sci. USA, 88, P 7328 (1991).
93- Goldbeter, A. , Proc Natl Acad. Sci USA , 88, P 9107 (1991).
94- Felix, M.A. , Labbe, J.C. , Doree, M., Hunt, T. and Karsenti, E., Nature, 346, P 379 (1990).
95- von Meyenburg, H.K. , in Bioogical and Biochemical Oscillators, B. Chance, E.K. Pye, T.K. Ghosh and B. Hess, Eds. , Acad. New York, P 411 (1973).
96- Fiechter, A., in Methods in Cell Biology II, D.M. Prescott, Ed., Acad. New York , P 97 (1975) .
97- Lee, K.J., Tribe, D.E. and Rogers, P.L., Biotechnol Lett., 1, P 421 (1979).
98- Parulekar, S.J., Semones, G.B., Rolf, M.J. , Lievense, M.J. and Lim, H.C. , Biotechnol. Bioeng., 28, P 700 (1986).
99- Porro, P., Martegani, E. , Ranzi, B.M. and Alberghina , L., Biotechnol. Bioeng., 32, P 411 (1988).
100- . Munch, T., Sonnleithner, B. and Fiechter, A., j. Biotechnol., 22, P 329 (1992).
101- Lee, K.J. , skotnicki, M.L., Tribe, D.E. and Rogers, P.L., Biotecnol Lett, 3, P291 (1981).
102-Jobses, I.M.L , Egberts, I.M.L. , Luyben, G.T.C., K. Ch. A.M. and Roels, J.A., Biotnol. Bioeng., , P 868 (1986).
103-Borzani, W., Griori, R.E. and Vairo, M.L.R. , Biotechnol Bioeng., 19, P 1363 (1977).
104- Nielsen, J. and Villadsen, J. , Bioreaction Engineering Principles, preprint (1996).
105- Hjortso, M. A. and Nielsen, J. , Chem. Eng. Sci. , 49, P 1083 (1994).

106-Sonnleitner, B.and Kappeli, O., Biotechnol. Bioeng. , 28, P 927 (1986).
107-Strassle, C., Sonnleither, B. and Fiecher, A , J. Biotechnol, 7, P 299 (1988).
108-Strassle, C. , Sonnleitner, B. and Fiecher, A. , J. Biotechnol. , 9, P 191 (1989).
109-Cazzador, L. and Mariani, L., Microbiol. Biotechnol., 29, P 198 (1988).
110-Cazzador, L., Mariani, L., Martegani, E. and Alberghina, L , Bioproc. Eng., 5, P 175 (1990).
111-Yano, T. and Koga, S. , J. Gen. Appl. Microbial , 19, P 97 (1973).
112-Elnashaie, S.S.E.H.., Al- Hddad, S.M., Ibrahim,G. and El-Shishini , S.S. , Los Alamos Conference (1996)
113- Ajbar, A. and Ibrahim G. , Mathl. Comput. Modelling, 25 no 10, P 9 (1997).
114- Ibrahim . , G. and Ajbar, A. , TESCH, (1997)
115- Ajbar, A. and Ibrahim G. , Mathl. Comput Modelling, 25 no 2, P31 (1997).
116- Guyton, A.C., in Medical Physiology, 7th ed., W.B. Saunders Company, P 137, 551-553, P 682 (1986).
117- Canovas- Munoz. M.D., Munoz- Delgado, E., and Vidal , C.J. , Biochem. Biophys. Acta. 1076, P 259 (1991)
118- Koch, A.L., J. Theor. Biol. ,P 120 (1986)..
119- Goldman, R., Silman, I. , Caplan, S.R. , Kedem, O. And Katchlski, E. , Science, 150, P 108 (1965).
120- Alcantara, F. And Monk, M. , J. Gen. Microbiol., 85, P 321 (1974)
120a- Elnashaie , S.S.E.H. , Ibrahim , G. and El-Refaie , M.A. , Appl. Biochem. Biotechnol. , 8 , P275 (1983)
120b- Elnashaie , S.S.E.H. , Ibrahim , G. and El-Refaie , M.A. , Appl. Biochem. Biotechnol. , 8 , P467 (1983)
120c- Elnashaie , S.S.E.H. , Ibrahim , G. and El-Shishini , S.S. , Appl. Biochem. Biotechnol. , 9 , P455 (1984)
121- Tomchik , K.J. and Devreotes , P.N. , Science , 212 , P443 (1981)
122- Gerisch , G. , Annu. Rev. Biochem. , 56 , P 853 (1987)
123- Martiel , J.L. and Goldbeter , A. , Biophys. J. , 52 , P 807 (1987)
124- Devreotes , P.N. and Sherring , J.A. , J Biol Chem. , 260 , P 6378 (1985)
125- Gundersen , R.E. , Johnson , R. , Lilly , P. , Pitt , G. , Pupillo , M., Sun , T. , Vaughan , R. and Devreotes , P.N. , in Cell To Cell Signalling : From Experiments to Theoretical Models , ed. A. Goldbeter , Acad. Press , London , P 477 (1989)
126- Goldbeter , A. and Martiel , J.L. , FEBS Lett. , 191 , P 149 (1985)
127- Martiel , J.L. and Goldbeter , A. , Nature , 313 , P 590 (1985)
128- Durston , A.J. , Dev. Biol. , 38 , P 308 (1974)
129- Coukell , M.B. and Chan , F.K. , FEBS Lett. , 110 , P 39 (1980)
130- Goldbeter , A. and Wurster , B. , Experientia , 45 , P 363 (1989)
131- Ibrahim , G. , TESCE , no 2 (1996)
132- Goldberger , A.L. and West , B. , Chaos in Biological Systems , eds. Holden , A.V. , Degn , H. and Olsen , L.F. , New York : Plenum , P 1 (1987)
133- Kaplan , D.T. , Furman , M.I. , Pincus , S.M. , Ryan , S.M. , Lipsitz , L.A. and Goldberger , A.L. , Biophys. J , 59 , P 945 (1991)
134- Skinner , J.E. , Neurol. Clin. , 11 , P 325 (1993)
135- Skinner , J.E. , Pratt , C.M. and Vybiral , T. , Am. Heart J ,125 , P 731 (1993)

136- Goldberger , A.L. , Rigney , D.R. and West , B. , Sci. Am. , 262 , P 42 (1990)
137- Grebogi , C. , Ott , E. and Yorke , J.A. , Phys. Rev. , A37 , P 1711 (1988)
138- Shinbrot , T. , Grebogi , C. , Ott , E. and Yorke , J.A. , Nature , 363 , P 411 (1993)
139- Plapp , B.P. and Hubler , A.W. , Phys. Rev. Lett. , 65 , P 2302 (1990)
140- Ott , E. , Grebogi , C. and Yorke , J.A. , Phys. Rev. Lett. , 64 , P 1196 (1990)
141- Guemez , J. and Matias , MA. , Phys. Lett. , A 181 , P 29 (1993)
142- Lima , B. and Pettini , M. , Phys. Rev. , A 41 , P 726 (1990)
143- Braimar , Y. and Goldhirsch , I. , Phys. Rev. Lett. , 66 , P 2545 (1991)
144- Li , Y.X. , Halloy , J. , Martiel J.L. , Wurster , B. and Goldbeter , A. , Experientia , 48 , P 604 (1992)
145- Halloy , J. , Li , Y.X. , Martiel , J.L. , Wuster , B. and Goldbeter , A. , Phys. Lett. , A 151 , P 33 (1990) and 159 , P 442 (1991)
146- Richter , P.H. and Ross , J. , Biophys. Chem. , 12 , P 285 (1980)
147- Li , Y.X. and Goldbeter , A. , Biophys. J. , 55 , P 125 (1989)
148- Goldbeter , A. and Li , Y.X. , in Cell To Cell Signalling , From Experiments to Theoretical Models , ed Goldbeter , A. , Acad. Press , London , P 415 (1989)
149- Knobil , E. , New England J Med. , 305 , P 1582 (1981)
150- Li , Y.X. and Goldbeter , A. , Biophys. J , 61 , P 161 (1992)
151- Goodwin , B.C. , Adv. Enzyme Regul. , 3 , P 425 (1965)
152- Walter , C. , J Theor. Biol. , 27 , P 259 (1970)