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MODELING A BIOCHEMICAL OSCILLATOR

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INTRODUCTION

Three kinds of oscillations have been observed in the peroxidase-oxidase reaction : simple, chaotic, and bursting (Olsen and Degn, 1977; see Fig. 1). A model of this reaction, proposed by Degn, Olsen, and Perram (1979), displays these kinds of oscillations in addition to what we call complex oscillations. Using a combination of numerical simulation and bifurcation analysis, our group is attempting to systematically analyze the progression of behavior that leads from stable steady states through simple and complex oscillations to chaos in the Degn-Olsen-Perram (DOP) model. We want to be able to predict where (i.e., at what rate constants) a given behavior (in particular, chaos) will be found in the model. This should aid in the understanding of the peroxidase-oxidase reaction and in the design of future experiments.

THE REACTION AND THE MODEL

Horseradish peroxidase catalyzes the oxidation of NADH by molecular oxygen in the reaction known as the peroxidase-oxidase (PO) reaction. The PO reaction is oscillatory in a semi-open, well-stirred system at acidic pH (pH 5.6 is commonly used). The semi-open system must have: 1) a constant, slow inflow of NADH (about 10 μL/hour); and 2) a constant supply of gaseous O_2 over the system (Degn, *et al.,* 1979). Oscillations occur over a variety of enzyme concentrations in the μM range. As can be seen in Figure 1, varying the enzyme concentration yields three different kinds of oscillations : 1) simple oscillations with a single amplitude (Fig. 1a); 2) chaotic oscillations with random mixtures of varying amplitudes (Fig. 1b); and 3) bursting oscillations with distinct quiet and active phases (Fig. 1c). In this paper, the transition from simple oscillations to chaos will be discussed. In another study (Aguda, *et al.,* 1989), bursting oscillations were discussed.

The DOP model was proposed in 1979 to account for the oscillations and other behavior found in the PO reaction. This model involves four species (O_2, NADH, and two intermediates) in an eight step mechanism and consists of the associated mass action kinetic equations (Figure 2). This model was studied by solving the system of four ordinary differential equations on a VAX8800 computer using an implementation of the Runge-Kutta-Verner method (IMSL routine DIVPRK).

The DOP model yields results qualitatively similar to those seen experimentally, and decreasing the rate constant k_1 controls the kind of oscillation observed much as decreasing the enzyme concentration does in the experimental system (Figure 3). The oscillations of mixed amplitude in the DOP model (e.g., Figure 3b), however, are not necessarily chaotic but sometimes have a definite, periodic pattern of large and small amplitudes. This type of oscillation is referred to as a "complex oscillation." The repeating patterns in complex oscillations are conveniently represented by the notation L^S, where L is the number of large amplitude

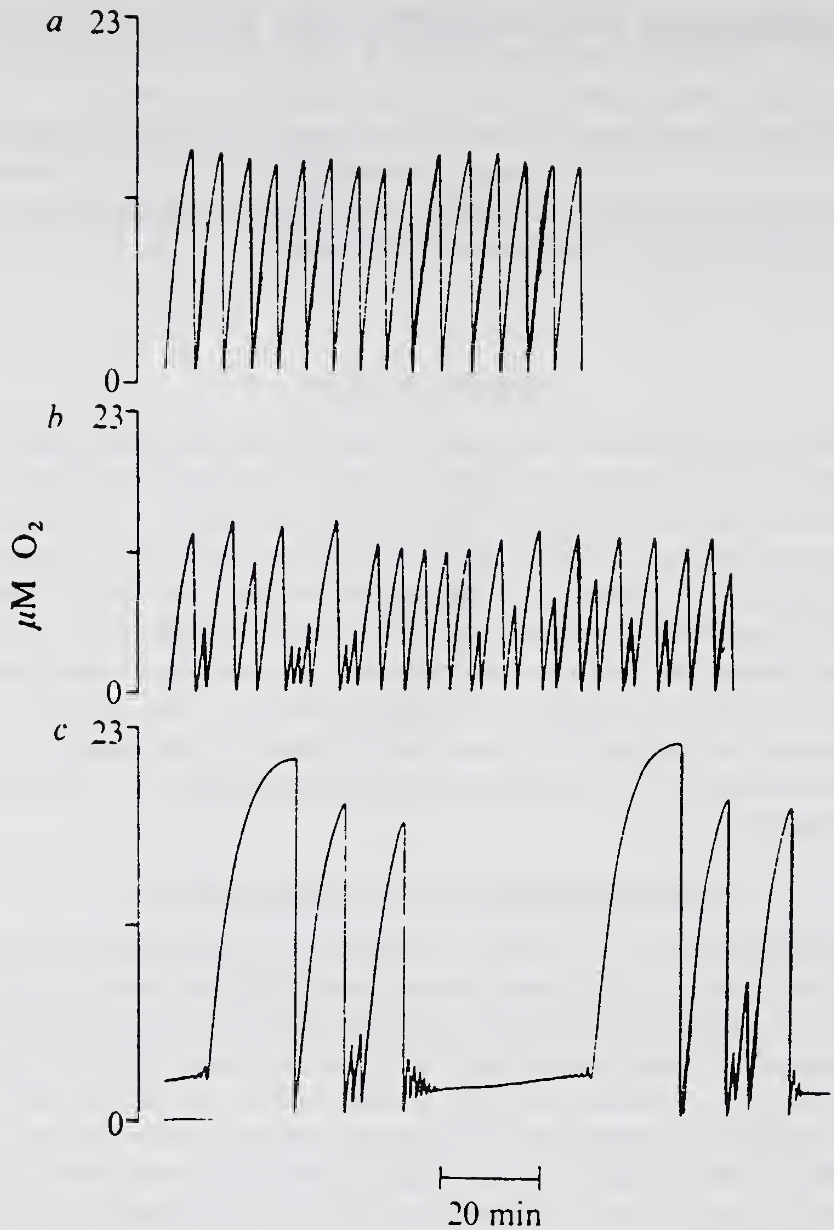

FIGURE 1. Experimental data from the peroxidase-oxidase reaction. The concentration of O_2 was measured with a Clark electrode. The shape of the oscillations was controlled by the concentration of the enzyme horseradish peroxidase. The enzyme concentrations were: a) 0.9 μM; b) 0.55 μM; and c) 0.45 μM.

oscillations, and S is the number of small amplitude oscillations. For example, the repeating pattern in Figure 3b is (4^2 3^3 4^4; this pattern has been confirmed with much longer calculations than the one shown). Complex oscillations have not yet been observed experimentally in the PO reaction; only *random* or *chaotic* mixtures of amplitudes have been seen experimentally, as in Figure 1b. The chaotic states seen in the DOP model occur at parameter values near those yielding complex oscillations (Larter, *et al.*, 1988), and these two types of behavior may be intimately related.

PHASE PORTRAITS AND BIFURCATIONS

Figures 1 and 3 are examples of time series. A time series represents a dynamical (time dependent) system by showing how one of the dependent variables changes with the independent variable, time. An alternative representation of

Degn-Olsen-Perram Model

Mechanism:

$$A + B + X \xrightarrow{k_1} 2X \qquad Y \xrightarrow{k_5} Q$$

$$2X \xrightarrow{k_2} 2Y \qquad X_0 \xrightarrow{k_6'} X$$

$$A + B + Y \xrightarrow{k_3} 2X \qquad A_0 \underset{k_{-7}}{\overset{k_7'}{\rightleftharpoons}} A$$

$$X \xrightarrow{k_4} P \qquad B_0 \xrightarrow{k_8'} B$$

Kinetic Equations:

$$\dot{A} = -k_1ABX-k_3ABY+k_7-k_{-7}A$$

$$\dot{B} = -k_1ABX-k_3ABY+k_8$$

$$\dot{X} = k_1ABX-2k_2X^2+2k_3ABY-k_4X+k_6$$

$$\dot{Y} = -k_3ABY+2k_2X^2-k_5Y$$

Where $k_6 = k_6'X_0$, $k_7 = k_7'A_0$, and $k_8 = k_8'B_0$, and A_0, B_0 and X_0 are constants.

FIGURE 2. The Degn-Olsen-Perram model. A is $[O_2]$, B is [NADH], and X and Y are the concentrations of two reaction intermediates.

the trajectory of a dynamical system is a phase portrait, in which two (or possibly more) dependent variables are plotted against each other. The plane (or higher dimensional space) in which a phase portrait is drawn is the system's phase space. Figure 4 schematically shows the relationship between time series and phase portraits for simple and complex oscillations. In either case, the trajectories in such a system trace out a closed curve. Note that Figure 4b is actually a simple (non-self-intersecting) closed curve in a three, or higher, dimensional phase space. A trajectory can never actually intersect itself in phase space (Arnol'd, 1973). Phase portraits are very useful for focusing on the qualitative behavior of a dynamical system.

The transition from stable steady states to chaos generally does not occur gradually but rather is punctuated by sudden, qualitative changes in behavior. A sudden change in either a mathematical model or a physical system is called a bifurcation (Guckenheimer and Holmes, 1983). One way to study a system's bifurcation behavior is to first set all of its parameters (in this case, the rate constants) so that the system is in a stable steady state and then to vary one of the parameters (now called the bifurcation parameter) while observing how the system's phase portrait changes as a function of the bifurcation parameter. Figure 5 schematically represents the changes in a system's phase portrait as the system undergoes two important kinds of bifurcations: the Hopf and the torus bifurcations.

A Hopf bifurcation occurs when a system's qualitative behavior changes from a stable steady state to simple oscillations (a supercritical Hopf bifurcation; Segal, 1980). A system's phase portrait signals a Hopf bifurcation by changing from a

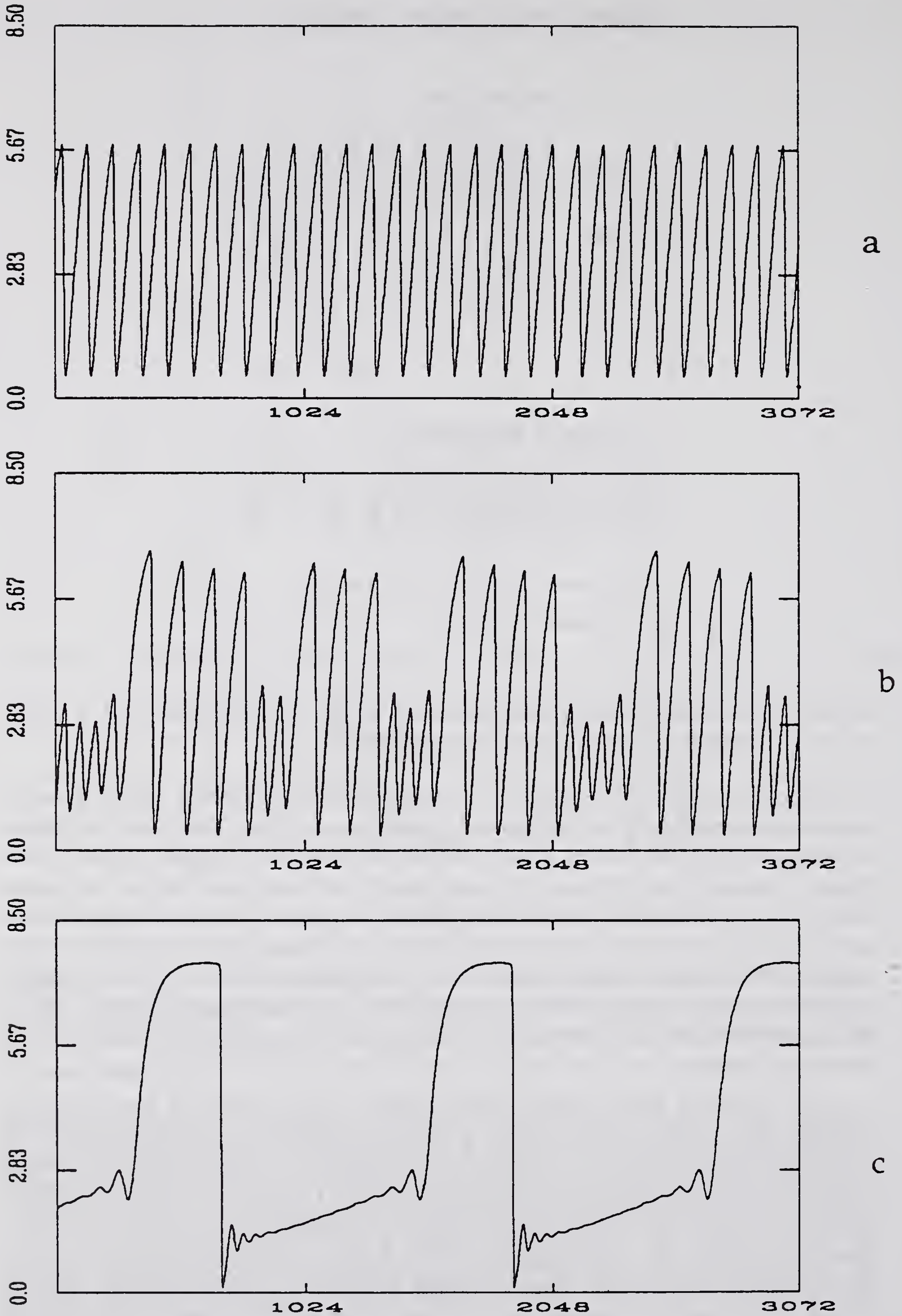

FIGURE 3. Calculated time series in A ($[O_2]$) from the DOP model. The shape of the oscillations was controlled by the value of the rate constant k_1. The values of k_1 were: a) 0.12; b) 0.9; and c) 0.048. The values of the other rate constants were: $k_2 = 1250$, $k_3 = 0.046875$, $k_4 = 20$, $k_5 = 1.104$, $k_6 = 0.001$, $k_7 = 0.89$, $k_{-7} = 0.1175$, and $k_8 = 0.5$. The time units are arbitrary.

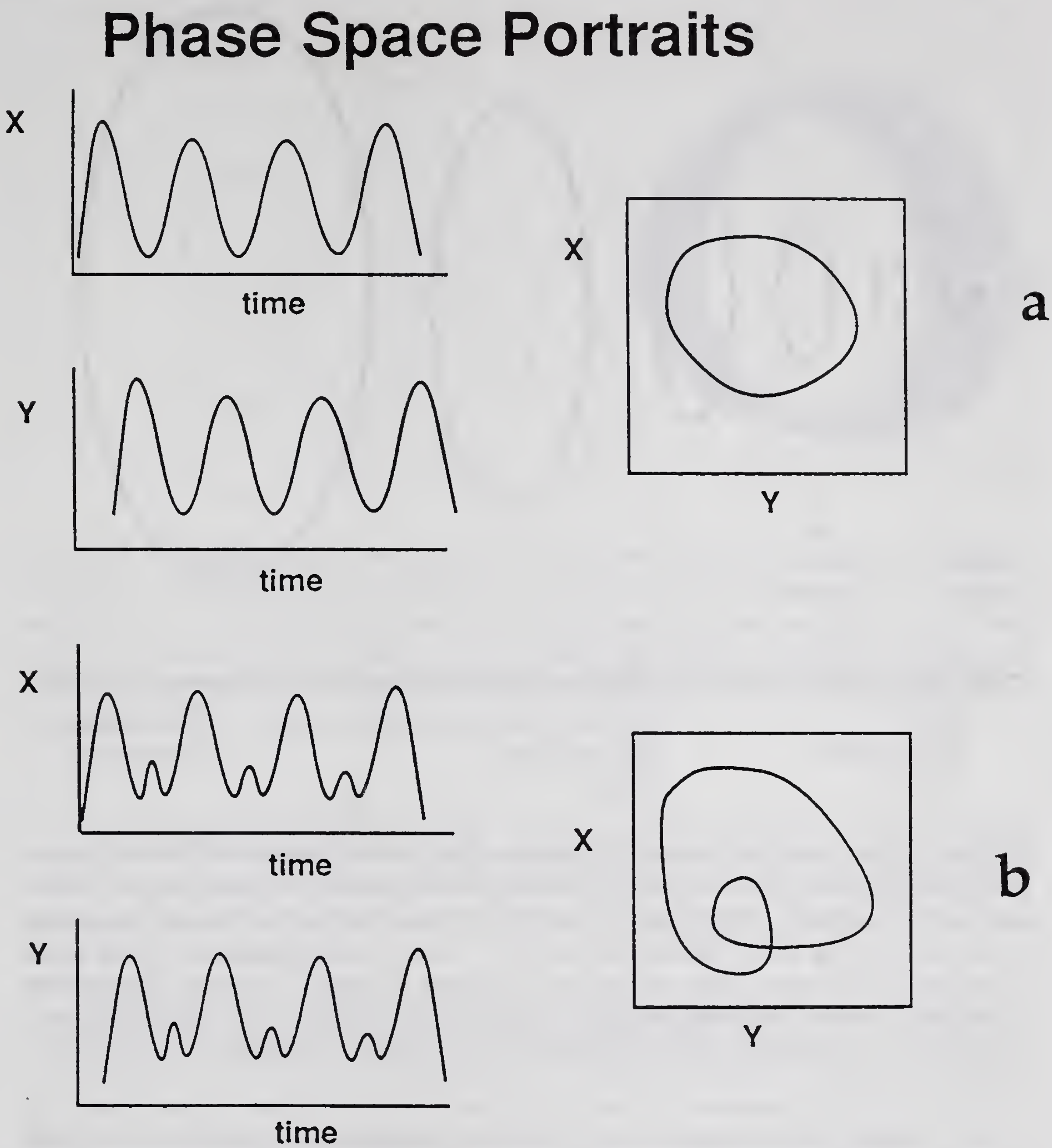

FIGURE 4. Schematic time series and phase portraits for simple and complex osciallations.

point to a simple closed curve called a limit cycle. The limit cycle grows as the the bifurcation parameter moves further from the Hopf bifurcation point, and its location in phase space may change as well. If a system has at least three degrees of freedom, it can undergo another bifurcation which gives rise to the three dimensional analog of a limit cycle: a torus (which is simply the surface of a doughnut). A torus bifurcation can be thought of as a second Hopf bifurcation and is technically known as a Naimark-Sacker bifurcation (Langford, 1983). Figure 6 shows a limit cycle and a torus actually calculated from the DOP model. The bifurcation parameter in Figure 6 is k_7. Notice that the trajectory covers only the surface of the torus and does not intrude into the interior.

As demonstrated in Figure 7a, a torus can be cut and folded into a flat sheet (Abraham and Shaw, 1984). Under some conditions, a trajectory can wind around

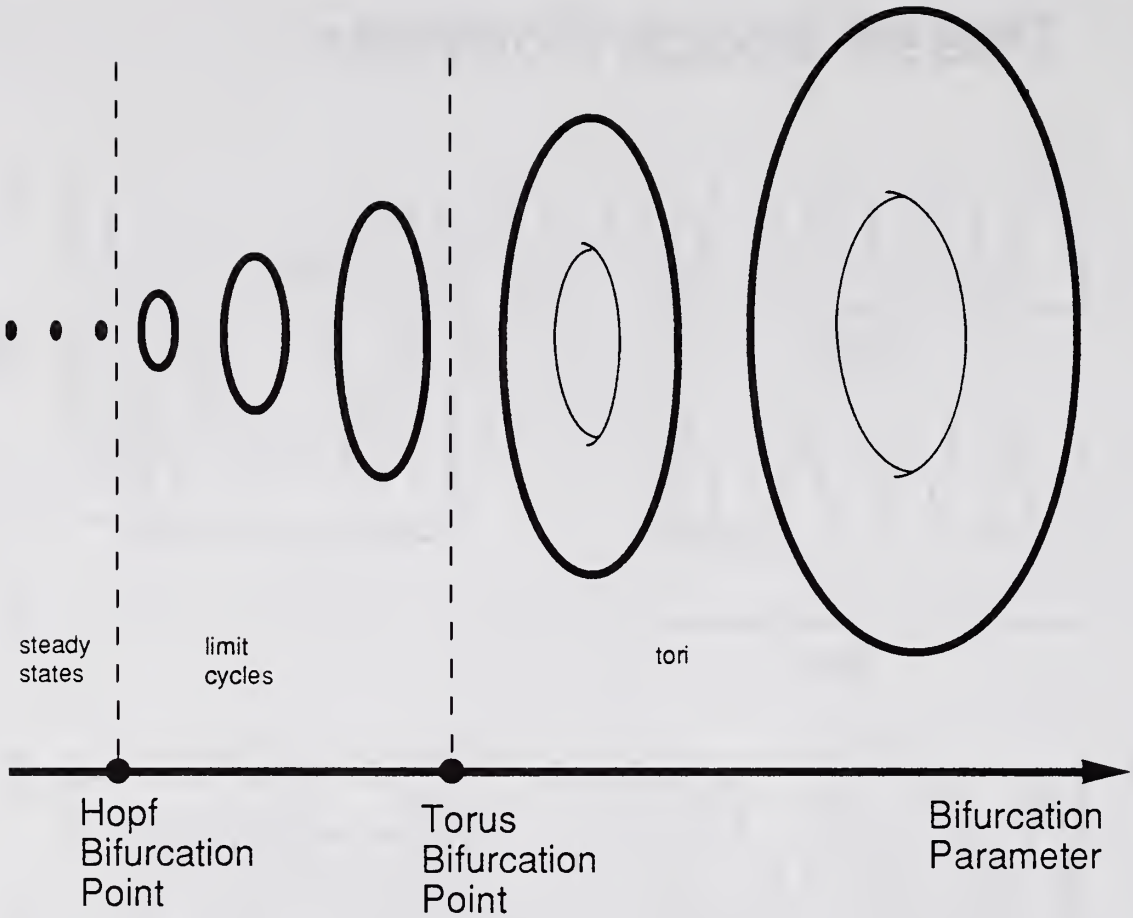

FIGURE 5. Schematic bifurcation diagram. The system starts (at the left) at a stable steady state. As the bifurcation parameter is tuned, the steady state moves, and then the system undergoes a Hopf bifurcation. As the bifurcation parameter is tuned past the Hopf bifurcation point, a limit cycle grows out of the (now unstable) steady state. The oscillations continue to grow, and then the system undergoes a second Hopf bifurcation, a torus bifurcation. Past the torus bifurcation quasiperiodic (see text) oscillations grow out of the (now unstable) limit cycle.

a torus forever without ever exactly retracing itself, and in the limit of infinite time, it will then densely cover the torus (or flat sheet). When a system densely covers the torus, it is called quasiperiodic (Figure 7b). Under other conditions, after one (or more) trips around the torus, a trajectory may begin to exactly retrace itself, so that it will *not* densely cover the torus. This latter behavior is called frequency locking (Figure 7c). The complex oscillations that have been observed in the DOP model (as well as other models of the PO reaction and other chemical oscillators) are examples of frequency locking; however, the quasiperiodic states that densely cover the torus have not been reported until now (nor had the torus bifurcation itself been identified in any model of the PO reaction). A quasiperiodic state and a nearby frequency locked state are shown in Figure 8 (Figure 6b is also an example of quasiperiodicity). These diagrams show clearly that the frequency locked state is a trajectory on the surface of a torus.

COMPLEX OSCILLATIONS AND CHAOS

Simple frequency locking does not account for many of the complex oscillations that we have found in the DOP model. Figure 10 shows two complex oscillations

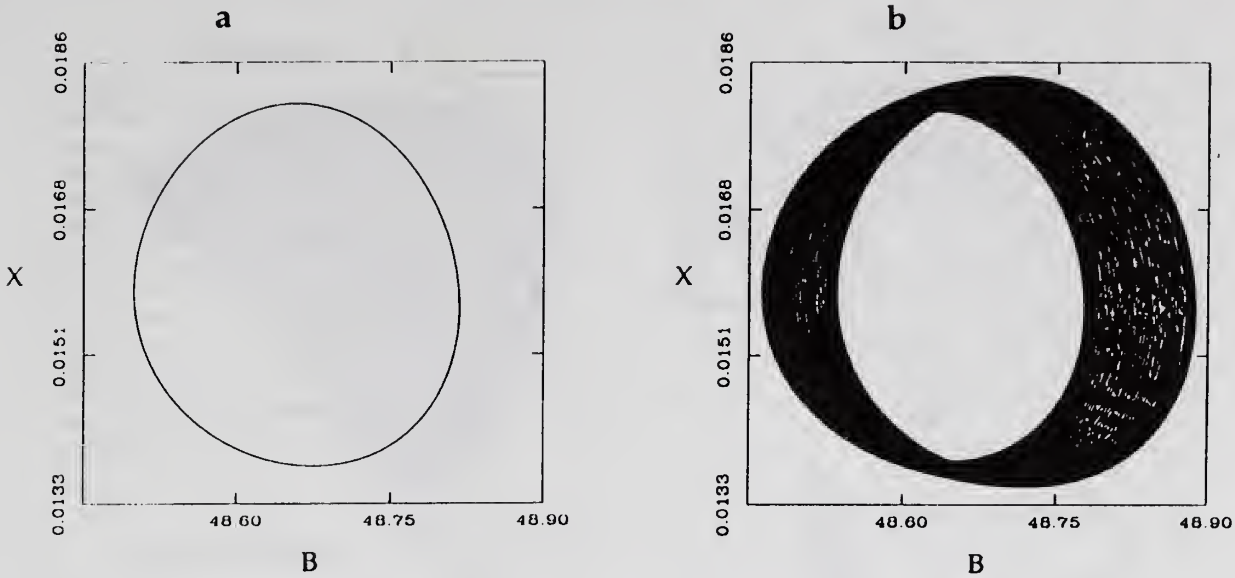

FIGURE 6. Phase portraits from the DOP model showing a limit cycle (6a) and a torus (6b). All rate constants except k_1 and k_7 are as in Figure 3, and in both 6a and 6b, $k_1 = 0.0761555$. The limit cycle in 6a was obtained with $k_7 = 0.2$. The torus in 6b was obtained with $k_7 = 0.15$. Notice how tightly wound the trajectory in 6b is (only one trip around the torus is shown). Also, the inner and outer diameters of the torus are nearly identical.

at very similar parameter values. The state in Figure 10a is 4^{10}, while that in 10b is $(4^9\, 4^{11})$. The intriguing thing about Figure 10b is that the small oscillations appear to go through two different sized holes. To cover such a doughnut (with two holes) with a simple torus requires the mathematical impossibility that the torus should intersect itself.

A possible explanation for Figure 10b is that the system has undergone a *third* Hopf bifurcation. The result of a third Hopf bifurcation would be a three-torus (Berge, *et al.*, 1984) as opposed to a two-torus, which is what we've been looking at up to now). A three-torus is a three dimensional surface of a *four dimensional* object, so the trajectory in Figure 10b could sit on (actually *in*) a three-torus without necessitating that the torus intersect itself. A third Hopf bifurcation is possible in the DOP model, because it has four variables and therefore a four dimensional phase space (up to this point, only two dimensional projections from this four dimensional space have been discussed).

A three-torus can be unfolded to a solid parallelepiped (a higher dimensional analog to Figure 7a cannot be drawn, since this would require four dimensions). On a flat sheet (i.e., a two-torus), only quasiperiodicity or frequency locking can occur, because the trajectory must be parallel to itself every time it goes around the torus (or across the sheet), otherwise it would intersect itself, which it cannot do. In a three-torus (a solid region), however, there is much more freedom of movement and the trajectory need not remain parallel to itself in order to avoid self intersection. So a new type of behavior that is neither quasiperiodic nor frequency locked is possible on a three-torus. This new type of behavior is chaos (Berge, *et al.*, 1984).

The chaotic states found in the DOP model occur at parameter values close to where complex oscillations of the type shown in Figure 10b occur. In a previous

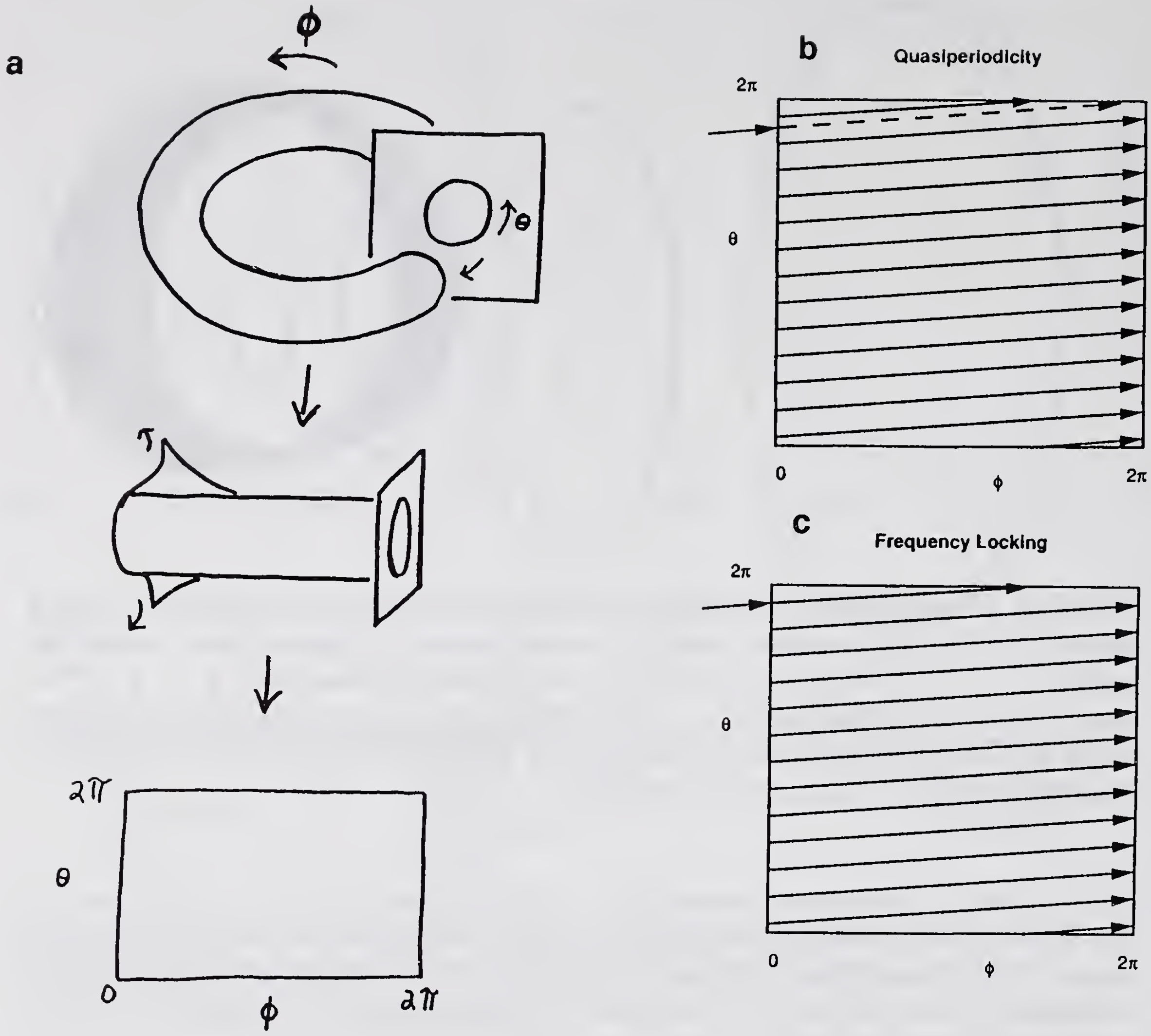

FIGURE 7. 7a shows how to turn a torus into a flat sheet. Since there are two independent angles of rotation on the torus, two edges of the sheet can be designated as axes, θ and ϕ, going from 0 to 2π. When either of the two angles reaches the value 2π, it is reset to 0, leaving the other angle unchanged. In other words, if a trajectory leaves the sheet at the top (left) it comes back on the bottom (right). 7b shows the path of a trajectory once around the torus (spread out on a sheet) in a quasiperiodic state and indicates that the trajectory will not retrace itself. Figure 7c shows a frequency locked state and indicates how it repeats itself every time it goes around the torus.

study, the complex oscillations in the DOP model were shown to form a devil's staircase structure (Larter, *et al.*, 1981). In this devil's staircase structure, parameter values at which complex oscillations of the type L^S (such as 4^{10}) occur are separated by parameter values at which combinations of the type $(L^S)_1$ $(L^S)_2$ . . . $(L^S)_n$, such as (4^9 4^{11}), occur. It seems that most of these combination states are periodic, that is, the combinations are regular. But chaotic states also exist, in which the combinations become random, as they do in the experimental system. A third Hopf bifurcation (the existence of which is still speculative) could explain the origin of both the regular and chaotic combinations as well as why they occur together in parameter space.

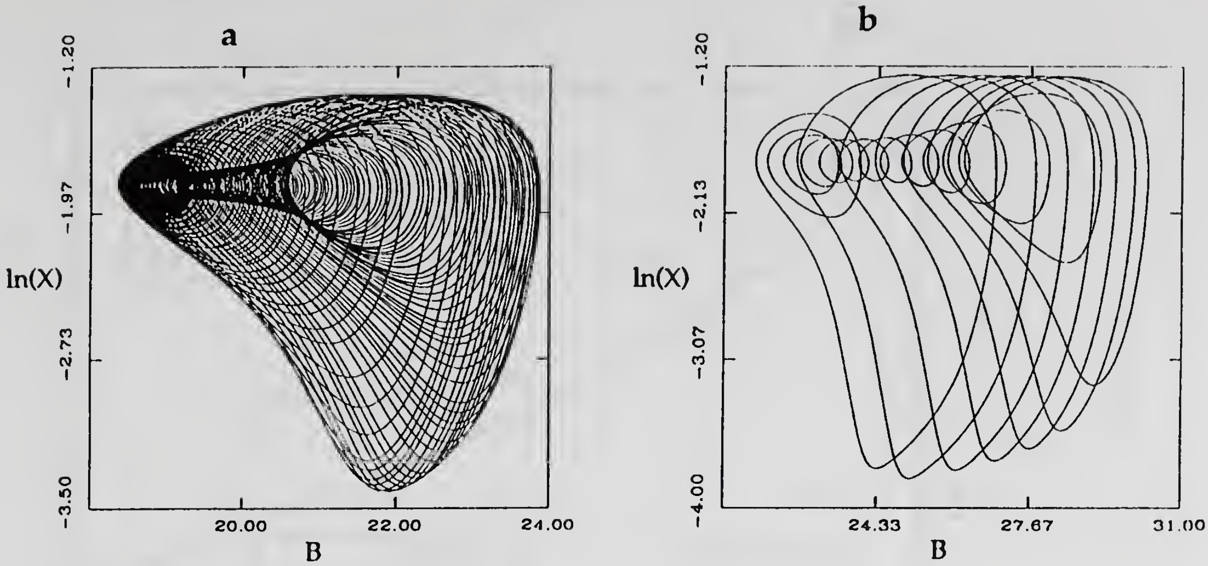

FIGURE 8. Phase portraits to illustrate frequency locking on a torus. A semilog scale is used both here and in Figure 10. All rate constants except k_1 and k_7 are as in Figure 3. In both 8a and 8b, $k_7 = 0.775$, while in 8a, $k_1 = 0.2$, and in 8b, $k_1 = 0.15$. Figure 8a is a quasiperiodic state that has already wrapped around the torus several times. Figure 8b is a frequency locked state. Notice that the torus has moved and changed shape somewhat between 8a and 8b.

CONCLUSION

The simple and complex oscillations in the DOP model arise from a Hopf and a torus bifurcation, respectively. Also, we now know (from using AUTO86) where these bifurcations occur, so that we can now predict accurately where simple and complex oscillations exist in the DOP model without doing time consuming and costly numerical simulations on a computer. Since the rate constants chosen in our bifurcation analysis correspond to experimentally manipulable parameters, this significantly enhances the predictive power of the DOP model. Additionally, quasiperiodic states have been formed, which previously were the "missing-link" in the transition from simple to complex oscillations in this model. Finally, we have shown how a third Hopf bifurcation, if it exists, could account for both the regular and chaotic combinations of complex oscillations that have been observed and that cannot be explained by simple frequency locking on a two-torus.

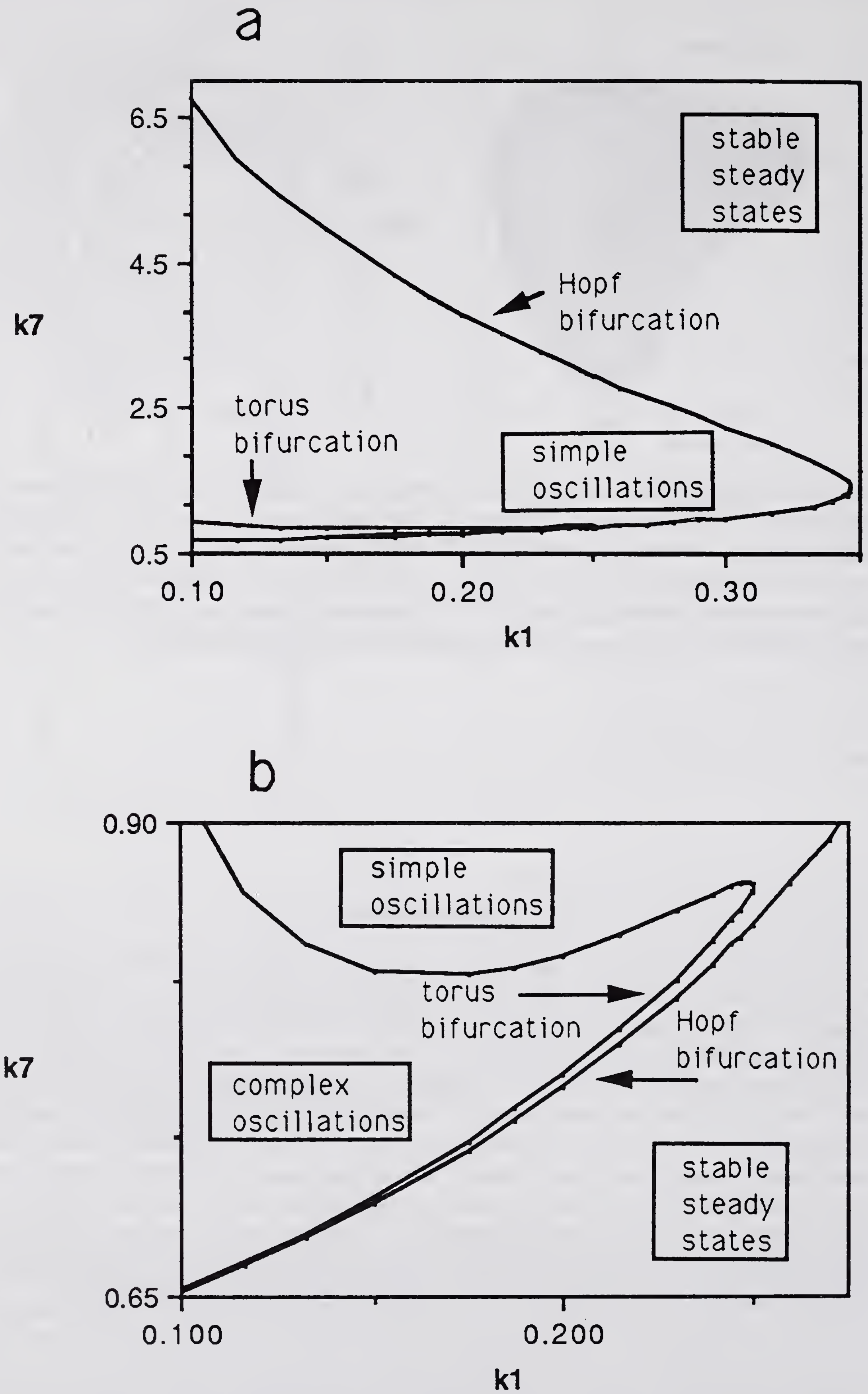

FIGURE 9. Two-parameter bifurcation diagram for the PO reaction. The two parameters are k_1 and k_7, as indicated on the axes. Figure 9b is a close-up of the part of 9a containing the torus bifurcation curve, which is barely visible in the lower left of 9a.

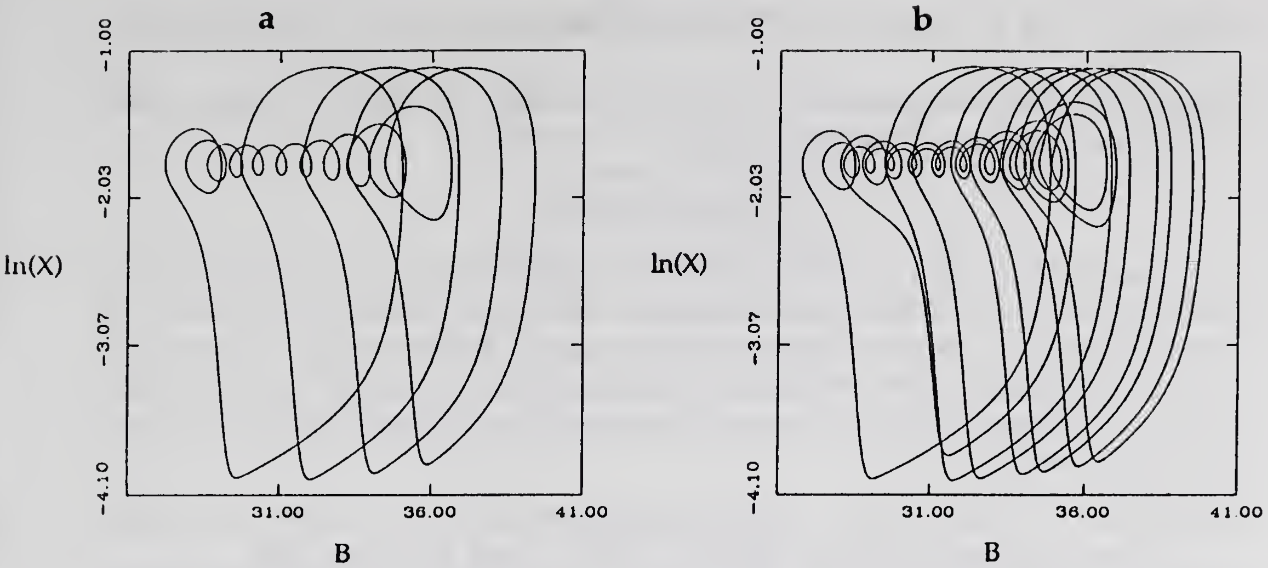

FIGURE 10. Phase portraits illustrating the two types of complex oscillations. 10a shows a 4^{10} state that can easily be explained by frequency locking on a two-torus. 10b shows a (4^9 4^{11}) state in which the smaller oscillations appear to trace out two differently sized holes.

LITERATURE CITED

Abraham, R.H. and C.D. Shaw. 1984. Dynamics—The geometry of behavior, part 1: Periodic behavior. Ariel Press, Santa Cruz, California.

Aguda, B.D., R. Larter, and B.L. Clarke. 1989. Dynamic elements of mixed-mode oscillations and chaos in a peroxidase-oxidase model network. J. Chem. Phys. 90: 4168-4175.

Arnol'd, V.I. 1973 Ordinary differential equations. The MIT Press, Cambridge, Massachussetts.

Berge, P., Y. Pomeau, and C. Vidal. 1984. Order within chaos. John Wiley and Sons, New York.

Degn, H., L.F. Olsen, and J.W. Perram. 1979. Bistability, oscillation, and chaos in an enzyme reaction. Ann. N.Y. Acad. Sci. 316: 623-637.

Doedel, E. 1986. Auto: Software for contination and bifurcation problems in ordinary differential equations. Pasadena, California.

Guckenheimer, J. and P. Holmes. 1983. Nonlinear oscillations, dynamical systems, and bifurcation of vector fields. Spring-Verlag, New York.

Langford, W.F. 1983. A review of interactions of Hopf and steady state bifurcation. *In:* G.I. Barenblatt, G. Ioos, and D.D. Joseph (Eds.), *Nonlinear Dynamics and Turbulence*, pp. 215-237, Pitman Publishing Limited, London.

Larter, R., C.L. Bush, T.R. Lonis, and B.D. Aguda. 1987. Multiple steady states, complex oscillations, and the devil's staircase in the peroxidase-oxidase reaction. J. Chem. Phys. 87(10): 5765-5771.

Larter, R., C.G. Steinmetz, and B.D. Aguda. 1988. Fast-slow variable analysis of the transition to mixed-mode oscillations and chaos in the peroxidase reaction. J. Chem. Phys. 89(10): 6506-6514.

Olsen, L.F. and H. Degn. 1977. Chaos in an enzyme reaction. Nature 267: 177-178.

Segel, L.A. 1980. Mathematical models in molecular and cellular biology. Cambridge University Press, Cambridge.

ADDENDUM

Since the time this work was submitted for publication, we have determined that the three-torus explanation suggested above as a possible mechanism by which chaos might arise in this model is not correct. Rather, the two dimensional torus goes through a "wrinkling" transition and breaks up into a fractal object which can support chaotic dynamics (Steinmetz and Larter, 1991; Larter and Steinmetz, 1991).

Steinmetz, C.G. and R. Larter. 1991. The quasiperiodic route to chaos in a model of the peroxidase-oxidase reaction. J. Chem. Phys. 94: 1388-1396.

Larter, R. and C.G. Steinmetz. 1991. Chaos via mixed-mode oscillations. Phil. Trans. Roy. Soc. 95A: 7948-7955.