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Mathematical analysis of activation thresholds in enzyme-catalyzed positive feedbacks: Application to the feedbacks of blood coagulation

(proteases/protease inhibitors)

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ABSTRACT **A hierarchy of enzyme-catalyzed positive feedback loops is examined by mathematical and numerical analysis. Four systems are described, from the simplest, in which an enzyme catalyzes its own formation from an inactive precursor, to the most complex, in which two sequential feedback loops act in a cascade. In the latter we also examine the function of a long-range feedback, in which the final enzyme produced in the second loop activates the initial step in the first loop. When the enzymes generated are subject to inhibition or inactivation, all four systems exhibit threshold properties akin to excitable systems like neuron firing. For those that are amenable to mathematical analysis, expressions are derived that relate the excitation threshold to the kinetics of enzyme generation and inhibition and the initial conditions. For the most complex system, it was expedient to employ numerical simulation to demonstrate threshold behavior, and in this case long-range feedback was seen to have two distinct effects. At sufficiently high catalytic rates, this feedback is capable of exciting an otherwise subthreshold system. At lower catalytic rates, where the long-range feedback does not significantly affect the threshold, it nonetheless has a major effect in potentiating the response above the threshold. In particular, oscillatory behavior observed in simulations of sequential feedback loops is abolished when a long-range feedback is present.**

Homeostatic systems of higher animals often involve complex systems that center on proteolytic enzymes. Examples include blood coagulation, inflammation, blood pressure regulation, fibrinolysis, and complement. The majority involve enzyme cascades, in which an enzyme (a protease in these systems) produced in one step generates another enzyme in the next step and then on to subsequent steps, and all require complex controls to moderate the response.

Although this report centers on proteolytic systems, enzyme cascades exist in other systems. An example is the cascade of kinases that is initiated by the stimulation of G-protein-linked hormone receptors. The controls in these cases, however, are rather different, in that many enzyme-transformation steps in metabolism are reversible; e.g., phosphorylation by kinases is reversible by the action of phosphatases. In contrast, the key property of proteases is that their formation from an inactive precursor (the zymogen) is an irreversible step. Because of this, protease inhibitors are usually required to inactivate the enzymes that have been formed. Major examples of inhibitors include antithrombin III (blood coagulation), α_2-plasmin inhibitor (fibrinolysis), and C1 inhibitor (complement). The importance of antithrombin III in controlling clotting, for instance, is clearly demonstrated by the high incidence of thrombosis in people who are partially deficient in this inhibitor (for a review of the coagulation system and its major controls, see ref. 1). Another interesting area in which a protease cascade and protease inhibitors are likely involved is the dorsal-ventral patterning system in embryogenesis (2).

In addition to inhibitory control, proteolytic systems are often controlled by negative feedbacks, in which product(s) of a system inactivate something required in an earlier step. In blood coagulation the major example is the system in which thrombin is responsible for generating a protease called activated protein C, which then inactivates two cofactors that are required for thrombin generation (factors Va and VIIIa, see below). Like antithrombin III deficiency, defects in this system cause thrombosis.

The third major family of controls, which are the focus of this report, are particularly common in blood coagulation but are also known in other systems. They are the positive feedbacks, in which an enzyme generated later in a cascade acts to enhance or accelerate its own formation. Fig. 1 shows a selected portion of the clotting system in simplified form, starting with the generation of factor IXa, a protease formed early in coagulation, and leading to the formation of thrombin, the final protease of coagulation. Thrombin is responsible for formation of the fibrin clot. Although there are just two enzymes formed in this section of the clotting cascade—factor Xa and thrombin—three positive feedbacks are involved in regulating their formation.

A Protease Cascade. Let us examine the cascade sequence in Fig. 1 more closely. The first stage is the generation of the factor IXa–VIIIa complex. This involves the conversion of a cofactor protein, factor VIII, to its active form, factor VIIIa, and then the binding of factor VIIIa to factor IXa, forming the enzymically active complex. In the simplified model, these are collapsed into a single activation step. Until factor VIII is activated, factor IXa, although technically already a protease, is essentially inactive. The factor IXa–VIIIa complex is the active proteolytic enzyme that acts on factor X, a protein that has no enzyme activity, to generate the enzyme factor Xa. The generation of factor VIIIa from factor VIII can be catalyzed by either the immediate product enzyme, factor Xa, or by the distant product enzyme, thrombin.

In the second stage of Fig. 1, factor Xa is the enzyme that acts on prothrombin to generate thrombin, but this too requires a positive feedback on a cofactor protein, factor V. Not until factor V is converted to its active form, factor Va, do we form the factor Xa–Va complex, which is the active enzyme complex. The major enzyme that activates factor V is thrombin, the immediate product of this reaction. Just as in the previous loop, the generation of the factor Xa–Va complex is collapsed into one step in the model.

If the enzymes of a positive feedback loop are not inactivated, the loop will always eventually go to completion, and all zymogens or precursors will finally be converted to their enzyme products. The lag phases will vary according to the

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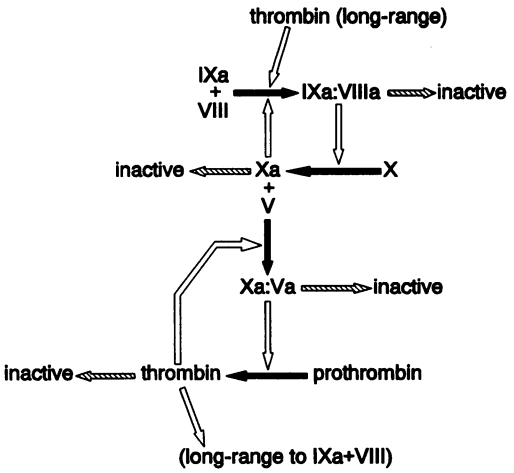

FIG. 1. Example of sequential feedback loops in blood coagulation. The main reactions involved between the initial appearance of the enzyme factor IXa and the generation of thrombin, the final enzyme, are shown. Arrows: open, the action of an enzyme in catalyzing the reaction pointed to; solid, the enzyme-catalyzed generation of an active enzyme product; cross-hatched, the inhibition or inactivation of an active enzyme. The long-range feedback action of thrombin is shown by the open arrow exiting at the bottom and entering at the top.

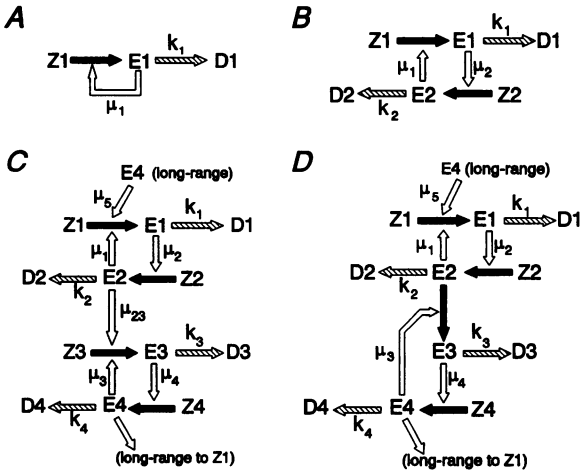

FIG. 2. Hierarchy of feedback-loop systems. Schemes A–D are illustrated. The coding of arrows is as described for Fig. 1. Zymogens (inactive precursors of enzymes) are shown as Z, active enzymes are shown as E, and inactivated (or "dead") enzymes are shown as D. Catalytic rate constants are $\mu = k_{cat}/(K_m + Z)$. Inhibition or inactivation rates are defined by first-order rate constants, k. Thus, in scheme A, $E1' = \mu \cdot E1 \cdot Z1 - k_1 E1$. To maintain the correspondence between schemes C and D, scheme D includes no Z3 species.

conditions and kinetic parameters but not the final enzyme yields (3). This behavior has recently been confirmed in both experimental observations and numerical simulation of coagulation in the absence of enzyme inhibitors (4, 5).

Normally, however, every active enzyme species—in Fig. 1, the IXa–VIIIa complex, factor Xa, the Xa–Va complex, and thrombin—is subject to irreversible inactivation of one type or another. The major mechanisms are (*i*) the action of inhibitors (e.g., antithrombin III), which irreversibly inhibit both factor Xa and thrombin; (*ii*) the spontaneous decay of factor VIIIa (6, 7); and (*iii*) the inactivation of the IXa–VIIIa and Xa–Va complexes by activated protein C, which we mentioned above. In this situation, analysis of the dynamic balance of enzyme generation and inactivation predicts a major property of feedback loops that include enzyme inhibition–threshold behavior (3).

Positive Feedback Systems. The coagulation example shown is but one of a hierarchy of positive-feedback systems, as shown in Fig. 2. In each of them, the activation or excitation of the system depends on the size of the stimulus exceeding a threshold value, and the response is nearly a step function of the size of the stimulus supplied. It is useful throughout to consider Z1, the initial zymogen or substrate species, as the initial stimulus. To get the system going, a trace of enzyme E1 must also be supplied.

Fig. 2, scheme A, shows the simplest feedback loop, where a product enzyme directly activates its own precursor. Although it is not included in Fig. 1, such "autolytic" feedback occurs in coagulation in the activation of the initiating complex of clotting, the tissue factor–factor VII complex, by its product enzyme, tissue factor–factor VIIa (8). It also exists in inflammation and *in vitro* coagulation, where the "contact" system involves the activation of factor XII (Hageman factor) by its direct product enzyme, factor XIIa (9).

Fig. 2, scheme B, shows a more complex situation in which two enzymes are generated by each other. As can be seen in Fig. 1, such loops exist in at least two places in coagulation. Analysis and numerical simulation of such a loop has been reported (3); it is included here to clarify the hierarchy.

The properties that arise when two feedback loops occur in sequence are considered next. In Fig. 2, scheme C, two loops are coupled by the product of the first loop (E2) catalyzing the initial step in the second loop (E3 formation). Although we are not aware that such a system exists in nature, this configuration of reactions is amenable to exact mathematical analysis, as we discuss below. It also serves to introduce a slightly different situation, which does not yield well to conventional mathematical treatment but closely approximates a scheme that does exist in nature, presented in Fig. 2, scheme D. In this we see that E2, the enzyme product of the first loop, serves two very different functions: (*i*) an enzyme catalyzing the feedback activation of Z1 in the first loop and (*ii*) a substrate or pseudozymogen in the second loop, being the precursor of the active enzyme E3. The general form of this coupled-loop system is based on the section of the coagulation cascade illustrated in Fig. 1.

In addition to the properties of systems involving sequential positive feedbacks, we also investigate, mathematically and by numerical analysis, the role of long-range feedbacks and their effect on the response. The prime long-range feedback in clotting is the activation of factor VIII by thrombin (10). This feedback spans two stages in the clotting cascade (Fig. 1) and is shown diagrammatically as the long-range action of E4 in activating Z1 (schemes C and D).

Although we focus in this paper on feedbacks in proteolytic cascades, threshold behavior is by no means unique to positive feedbacks nor even to enzyme systems. The key feature is the kinetic balance of response generation and its decay or inhibition. For example, the density-dependent growth of algae is inhibited by grazers, but if the cell division rate exceeds a certain threshold, cell counts will suddenly surge before declining again (11). For neuron firing, if a threshold voltage is exceeded in an otherwise quiescent system, an action potential is initiated that manifests itself as a large excursion in membrane potential (12). In infectious diseases, the population of infectives is inhibited by death and recovery, but there is a threshold density that initiates an epidemic that is dependent on the intrinsic infection rate (13). Although the details obviously differ, there are nonetheless clear parallels between the general kinetic architecture of such systems and the positive feedbacks of enzyme cascades.

In the section below, *Analysis*, we analyze threshold conditions for activation of the systems illustrated in schemes A–C. In particular, we will see the significance of a long-range feedback in activating an otherwise subthreshold system. After this, in *Numerical Simulations*, scheme D is subjected to a range of computational trials in an attempt to assess the validity of the

mathematical results obtained for the more artificial model in scheme C. It will be seen that the general conclusions about long-range feedback are confirmed, with some additional and surprising insights into a possible additional role in proteolytic cascades.

Analysis

Reaction Kinetics. In a simple enzyme-catalyzed reaction, the rate of conversion of a substrate species Z by an enzyme E is generally a hyperbolic function of the concentration of Z. Let prime (′) denote differentiation with respect to time, $Z' = -k_{cat}E{\cdot}Z/(K_m + Z)$, where k_{cat} is the catalytic rate constant for the reaction, K_m is the Michaelis constant, and the concentrations of enzyme and substrate are shown by italic type. (We use Z because the precursors of proteolytic enzymes, the substrates in these models, are known as zymogens.) It should be pointed out that this Michaelis–Menten formulation is violated in a feedback system in which a feedback-activating enzyme exceeds the concentration of its substrate. We emphasize, however, that our focus is on the threshold conditions that lead to the initial onset of activation, rather than the subsequent dynamics of bulk enzyme generation.

We assume that the various active enzyme species, E, are inactivated or decay in first-order fashion, $E' = -k{\cdot}E$. The products of enzyme inactivation or decay are denoted by D (mnemonic for "dead", or inactive, enzyme). In natural systems, first-order kinetics are commonly found when an irreversible inhibitor is responsible, and examples have already been mentioned.

Concentrations and Units. Although much of the analysis is valid for any system, models have been set up to correspond approximately with known physiological cascades, in which the concentrations of the precursor (substrate) species increase from top to bottom, and stimulus amplification is a key feature. The units of concentration and time in both analysis and numerical simulations are arbitrary; although a very rough approximation to the coagulation system may be obtained if the concentration unit is assumed to be nanomolar, and the time unit is a minute.

Autolytic Feedback: Scheme A. The simplest feedback loop is an enzyme E1 that catalyzes its own formation from a substrate Z1, with inhibition of E1 leading to D1. In accordance with the assumptions made above, the differential equations that model this are

$$E1' = k_{cat}E1{\cdot}Z1/(K_m + Z1) - k_1E1$$
$$Z1' = -k_{cat}E1{\cdot}Z1/(K_m + Z1). \quad [1]$$

We may linearize Eq. **1** about the equilibrium $E1 = 0$, $Z1 = Z1_0$, where $Z1_0$ is the initial concentration of Z1. This reduces to the single equation

$$E1' = \mu_1 Z1_0 E1 - k_1 E1, \quad [2]$$

in which μ_1 is the constant $k_{cat}/(K_m + Z1_0)$. As long as an initial trace of E1 is provided to initiate the loop, E1 is evidently generated—the system fires, or ignites—if the following threshold condition is met:

$$\theta = \mu_1 Z1_0/k_1 > 1. \quad [3]$$

Otherwise $E1$ decays to zero without first increasing. A similar approach will be utilized below as we extend this simple prototype to more complicated feedback loops.

Scheme B. The reactions in scheme B can be similarly expressed by the following differential equations:

$$E1' = k_{cat,1}E2{\cdot}Z1/(K_{m,1} + Z1) - k_1E1,$$
$$E2' = k_{cat,2}E1{\cdot}Z2/(K_{m,2} + Z2) - k_2E2,$$
$$Z1' = -k_{cat,1}E2{\cdot}Z1/(K_{m,1} + Z1),$$
$$Z2' = -k_{cat,2}E1{\cdot}Z2/(K_{m,2} + Z2).$$

These equations are linearized about the equilibrium state defined by $Z1 = Z1_0$, $Z2 = Z2_0$ (the initial values of $Z1$ and $Z2$) and $E1 = E2 = 0$, to obtain the equations

$$E1' = \mu_1 Z1_0 E2 - k_1 E1$$
$$E2' = \mu_2 Z2_0 E1 - k_2 E2. \quad [4]$$

As in the previous scheme A, the constants μ_i are $k_{cat,i}/(K_{m,i} + Zi_0)$ for $i = 1,2$. The eigenvalues of this linear system are

$$\lambda_{1,2} = -\frac{(k_1 + k_2)}{2} \pm \left[\left(\frac{k_1 - k_2}{2}\right)^2 + \mu_1\mu_2 Z1_0 Z2_0\right]^{1/2}. \quad [5]$$

λ_2 is always negative but the first eigenvalue λ_1 is positive whenever the threshold condition

$$\theta = (\mu_1\mu_2 Z1_0 Z2_0)/(k_1k_2) > 1 \quad [6]$$

is satisfied. Indeed, the product of the eigenvalues is negative if and only if Eq. **6** holds, and in this case the origin of the $E1,E2$ plane is an unstable equilibrium. This condition is the analogue of the corresponding excitation criterion for scheme A.

A glance at the actual solutions, for initial values $E1_0 > 0$ and $E2_0 = 0$, would show that $E1$ quickly decays to zero when Eq. **6** is violated, whereas a small amount of E2 is initially generated (by the initial trace of E1) before it too goes to zero. On the other hand, when Eq. **6** is satisfied, there is an initial small dip in E1, caused by the lag in E2 formation before feedback formation of E1. By analyzing this model further, one can obtain expressions for the total yields of E1 and E2—namely, the amount of substrate that is converted (3).

Scheme C. Next we consider the extended reaction system in scheme C. This begins to approach the actual feedback cascade seen in scheme D. Included here is the possibility of long-range activation in which E4 feeds back to generate E1 from Z1.

The linearized equations that correspond to scheme C are obtained in the same manner as in the previous models by perturbing about the equilibrium state in which $Ei = 0$ and Zi are at their initial values for $i = 1, \ldots 4$. This leads to

$$E1' = \mu_1 Z1_0 E2 - k_1 E1 + \mu_5 Z1_0 E4,$$
$$E2' = \mu_2 Z2_0 E1 - k_2 E2,$$
$$E3' = \mu_{23} Z3_0 E2 - k_3 E3 + \mu_3 Z3_0 E4,$$
$$E4' = \mu_4 Z4_0 E3 - k_4 E4. \quad [7]$$

The Jacobian of the linearized system (Eq. **7**) is

$$\mathbf{J} = \begin{pmatrix} -k_1 & \mu_1 Z1_0 & 0 & \mu_5 Z1_0 \\ \mu_2 Z2_0 & -k_2 & 0 & 0 \\ 0 & \mu_{23} Z3_0 & -k_3 & \mu_3 Z3_0 \\ 0 & 0 & \mu_4 Z4_0 & -k_4 \end{pmatrix}, \quad [8]$$

and the characteristic equation for $\mathbf{J}$ is a quartic polynomial,

$$f(\lambda) = [(\lambda^2 + \lambda(k_1 + k_2) + (k_1k_2 - \mu_1\mu_2 Z1_0 Z2_0)]$$
$$\times [(\lambda^2 + \lambda(k_3 + k_4) + (k_3k_4 - \mu_3\mu_4 Z3_0 Z4_0)] - C = 0, \quad [9]$$

where C is the constant $\mu_2\mu_{23}\mu_4\mu_5 Z1_0 Z2_0 Z3_0 Z4_0$.

Consider first the case of no long-range feedback, when $C = 0$. It is readily seen that all eigenvalues are real and that the only possibility for a positive eigenvalue hinges on the two

factors in Eq. **9**, the first of which is the characteristic polynomial for the single-loop model treated earlier. Thus the double-loop system is activated if the threshold condition (Eq. **6**) is valid or if a similar condition is valid for the second loop:

$$\theta_1 = \frac{\mu_1\mu_2 Z1_0 Z2_0}{k_1k_2} > 1 \text{ or } \theta_2 = \frac{\mu_3\mu_4 Z3_0 Z4_0}{k_3k_4} > 1. \quad [10]$$

To exhibit the impact of long-range feedback, we now assume that all the eigenvalues are negative when $C = 0$ or, to put it another way, that $\theta_1,\theta_2 < 1$. When $C > 0$, the polynomial is shifted downward, and ultimately, when C is large enough, there will be a positive root, which demonstrates that activation is possible through long-range feedback (the system is rendered unstable) even if the system is stable (i.e., subthreshold) without feedback.

A necessary and sufficient condition that C be just large enough is that $f(\lambda)$ have one positive and three negative real eigenvalues, or one positive and one negative real with two complex eigenvalues. This requires the constant term in $f(\lambda)$ to be negative and it leads to the threshold condition

$$\theta = \frac{\mu_1\mu_{23}\mu_4\mu_5 Z1_0 Z2_0 Z3_0 Z4_0}{(k_1k_2 - \mu_1\mu_2 Z1_0 Z2_0)(k_3k_4 - \mu_3\mu_4 Z3_0 Z4_0)} > 1. \quad [11]$$

Note that Eq. **11** is satisfied when μ_5 is large enough even if neither threshold condition separately (Eq. **10**) is met. If only one of the conditions in Eq. **10** is true, then the inequality in Eq. **11** is reversed. Incidentally, the denominator in Eq. **11** can be simplified by observing that it is simply the product of the eigenvalues in the case when μ_5 is zero.

By computing the appropriate eigenvectors corresponding to Eq. **9**, the solution to Eq. **7** is exhibited in vector notation as **Au** where **A** is a matrix of the form

$$\mathbf{A} = \begin{pmatrix} a_{11} & a_{12} & 0 & 0 \\ a_{21} & a_{22} & 0 & 0 \\ a_{31} & a_{32} & a_{33} & a_{34} \\ a_{41} & a_{42} & a_{43} & a_{44} \end{pmatrix}$$

with all $a_{i,j}$ nonzero, and **u** is a vector whose components are exponentials in $\lambda_i t$. This shows that the activation threshold, $\theta_2 > 1$, for the second loop is always met whenever $\theta_1 > 1$, but the reverse is not necessarily true. Thus, the first loop will always ignite the second loop, but not always vice versa.

Similar conclusions can be reached regarding the double loop in scheme D. However, linearization for this more complex model, in which E2 plays the role of an active enzyme in the first feedback loop but is the inactive precursor—or pseudozymogen—of E3 in the second, obscures the role of a long-range feedback. To circumvent this difficulty, we utilized a numerical simulation of the corresponding differential equations for model D and showed that here too an otherwise stable double loop can be excited in the presence of long-range feedback. This is taken up in the next section.

To infer something of the possible behavior of E3 when E2 assumes the surrogate role of zymogen, we isolated out the second loop, allowing E2 now to be a time varying coefficient in a model that only generates E3 and E4. An initial phase of the reaction is considered during which E2 is still in a growth mode as a result of activation in the first loop, with a roughly linear rise in concentration, and by assuming Z4 to be in excess over E2 in this early phase so that it can be considered initially a constant $Z4_0$. This provides the equations

$$E3' = \mu_3 E2{\cdot}E4 - k_3E3,$$
$$E4' = \mu_4 Z4_0 E3 - k_4E4,$$
$$E2' = s - \mu_3 E2{\cdot}E4, \quad [12]$$

where s is the rate of E2 input from the first loop. This system has equilibria $E3 = s/k_3$, $E4 = s/\mu_3 c$, and $E2 = c$, where $c = k_3k_4/\mu_3\mu_4 Z4_0$ and the characteristic equation for the Jacobian of the linearized equations about this equilibrium is the cubic

$$\lambda^3 + \lambda^2(k_3 + k_4 + s/c) + \lambda s(k_3 + k_4)/c + k_3k_4 s/c = 0.$$

All the coefficients of the polynomial are positive and an examination of the discriminant reveals a range of s values for which there are one real and two complex roots in the left-half plane. In this case, the solutions decay in an oscillatory fashion. The numerical simulations of the next section verify this kind of response can occur in the absence of a long-range feedback.

Numerical Simulations

To this point the analysis has centered on the determination of the threshold conditions for each of the systems considered, i.e., the question of whether a system will initially fire. Numerical simulation allows us not only to examine the threshold question but also to go further and examine the later time courses of enzyme generation. For the purposes of this theoretical study, we simplify simulation by assuming that each enzyme-catalyzed reaction is described by simple second-order, rather than Michaelis–Menten, kinetics; i.e., $E' = \mu{\cdot}Z{\cdot}E$, where $\mu = k_{cat}/K_m$ (cf. Eq. **1** *et seq.*). This is valid for $K_m >> Z,E$.

Schemes C and D were solved numerically by using Gear's method for stiff systems (DIFSUB; ref. 14), with a per-step tolerance, $\varepsilon \leq 10^{-6}$, and double precision throughout.

Scheme C. The differential equations for scheme C are as follows:

$$Z1' = -(\mu_1 E2 + \mu_5 E4)Z1, \quad E1' = (\mu_1 E2 + \mu_5 E4)Z1 - k_1E1,$$
$$Z2' = -\mu_2 E1{\cdot}Z2, \quad E2' = \mu_2 E1{\cdot}Z2 - k_2E2,$$
$$Z3' = -(\mu_{23} E2 + \mu_3 E4)Z3, \quad E3' = (\mu_{23} E2 + \mu_3 E4)Z3 - k_3E3,$$
$$Z4' = -\mu_4 E3{\cdot}Z4, \quad E4' = \mu_4 E3{\cdot}Z4 - k_4E4.$$

The long-range feedback of E4 on Z1 is defined by the $\mu_5 E4$ term in the expressions for $Z1'$ and $E1'$. The two feedback loops of this model are linked at the activation of Z3 by E2, which is defined by the $\mu_{23}E2$ term in the expressions for $Z3'$ and $E3'$. Initiation of the system requires that either E1 or E2 is initially supplied. In all simulations, a trace of E1 is provided ($E1_0 > 0$), so that $E1_0/(E1_0 + Z1_0) = 0.001$. It is pertinent to note that at least in blood coagulation, it has been clearly shown that trace, or idling, levels of clotting enzymes are always present in the blood plasma (15).

Scheme D. Just as in scheme C, an initial trace level of E1 is provided so that $E1_0/(E1_0 + Z1_0) = 0.001$. To initiate the second feedback loop of scheme D, some E3 must similarly be either initially provided or generated directly (not by feedback). Of various possible means available, we have specified that E1, in addition to catalyzing the formation of E2, also generates E3, albeit at a much lower rate. The ratio of these rates of E3 and E2 generation by E1 is defined below by the constant C, which in all our simulations equaled 0.001. In physiological terms, this is equivalent to saying that E2, when generated, possesses a trace level (0.1%) of E3 activity.

The differential equations for scheme D are then

$$Z1' = -(\mu_1 E2 + \mu_5 E4)Z1,$$
$$E1' = (\mu_1 E2 + \mu_5 E4)Z1 - k_1E1,$$
$$Z2' = -\mu_2(1 + C)E1{\cdot}Z2,$$
$$E2' = \mu_2 E1{\cdot}Z2 - \mu_3 E4{\cdot}E2 - k_2E2,$$

$$E3' = \mu_2 C{\cdot}E1{\cdot}Z2 + \mu_3 E4{\cdot}E2 - k_3 E3,$$

$$Z4' = -\mu_4 E3{\cdot}Z4,$$

$$E4' = \mu_4 E3{\cdot}Z4 - k_4 E4.$$

Results. Analysis of scheme C predicted that under certain parameter conditions, the long-range reaction ($\mu_5 > 0$) may permit the excitation of an otherwise subthreshold system (Eq. **11**), and this has been confirmed by numerical simulation for stimulus sizes close to the threshold (Fig. 3). In this section, we wished to determine whether the same behavior holds for the more complex scheme D.

Numerical simulation of scheme D confirms that, just as in scheme C (Eq. **11**), the threshold for the first loop controls the threshold of the system. Under the particular combination of parameter values and initial conditions we have studied, excitation of the first loop (E2 formation) always leads to excitation of the second (E4 formation), just as in scheme C (data not shown). Numerical solution also shows that, as in scheme C, large μ_5 values in scheme D can enable activation of the system, but only when the stimulus, *Z1*, is close to the threshold. For more feasible lower values of μ_5, the existence of a long-range feedback does not significantly lower the threshold of a system.

A more interesting consequence of long-range feedback action as seen in Fig. 4*A* shows the generation of E4 at various levels of the initial stimulus Z1 in the absence of any long-range action of E4 on Z1 ($\mu_5 = 0$). At and below the threshold, no significant E4 is generated. However, above the threshold, a cycle of oscillation occurs in *E4* before the response finally decays. In contrast, when there is a long-range feedback ($\mu_5 > 0$; Fig. 4*B*), only one sharp spike of E4 generation is observed; and this significant change in the form of the response is observed even at low μ_5 values, where the threshold itself is essentially unaffected. While other mechanisms may be involved, the shift from oscillatory to nonoscillatory behavior may be a result of the increased rate of E2 generation that occurs when $\mu_5 > 0$, as was suggested by the theoretical analysis of scheme D.

Although we have not attempted quantitative simulation of the corresponding section of the clotting system (Fig. 1), it is useful to relate the present studies of model systems to coagulation. It has been known for some time that factor Xa can activate factor VIII, and in both pure experimental systems and numerical simulations, this enables the firing of the first loop in Fig. 1 (3, 16–18). Experiments in whole blood plasma systems, however, have shown that significant generation of thrombin by the second loop is almost entirely dependent on the activation of factor VIII by thrombin (19). In Fig. 4, we see that such a result is feasible, since even when both feedback loops are excited, peak E4 generation in the absence of long-range feedback may be relatively small and diffuse in time. In contrast the peak yield of E4 is strongly potentiated by the long-range feedback. To the extent that scheme D mimics an actual cascade (Fig. 1), we know that as long as the threshold of the first loop is exceeded, long-range feedback will ensure that activation takes place rapidly and decisively.

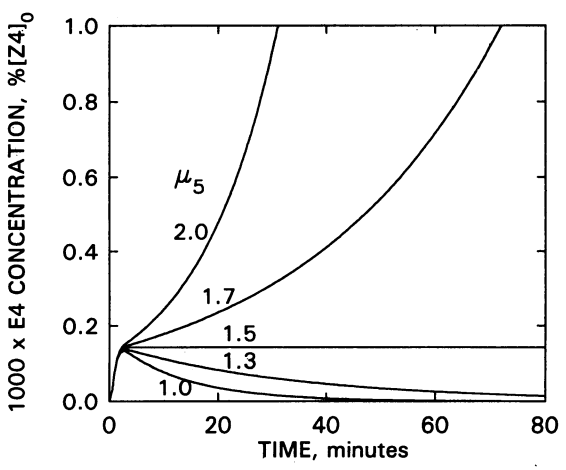

FIG. 3. Numerical simulation of scheme C: the generation of E4 at various rates of long-range feedback, μ_5 (k_{cat}/K_m, see *Numerical Simulations*). Initial concentrations and parameter values were as follows: $Z1_0 = 0.5$; $Z2_0 = Z3_0 = 10$; $Z4_0 = 100$; $\mu_1 = 1$; $\mu_2 = \mu_{23} = \mu_3 = \mu_4 = 0.1$; $k_1 = k_2 = 1$; $k_3 = k_4 = 5$; μ_5 was varied as shown. The predicted threshold under these conditions occurs at $\mu_5 = 1.5$.

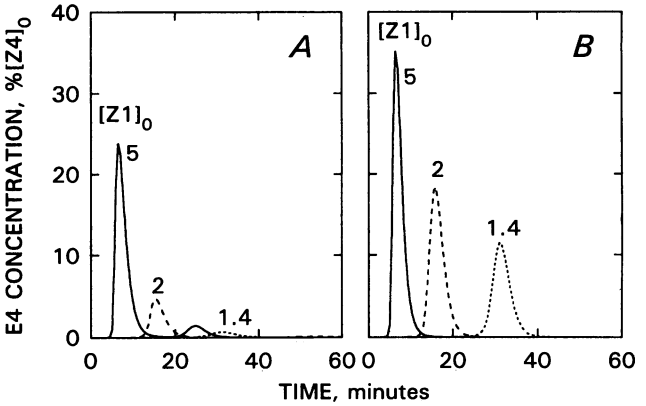

FIG. 4. Numerical simulation of scheme D: the generation of E4 at various rates of long-range feedback, μ_5, and stimulus size, $Z1_0$. Fixed initial concentrations and parameter values were as follows: $Z2_0 = 10$; $Z4_0 = 100$; $\mu_1 = \mu_3 = \mu_4 = 1$; $\mu_2 = 0.1$; $k_1 = k_2 = k_3 = k_4 = 1$. $Z1_0$ was varied as indicated, at μ_5 values of 0 (*A*) and 0.1 (*B*). The predicted threshold under these conditions occurs at $Z1_0 = 1$.

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