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Complex dynamics in a minimal model of the alternative pathway of the complement system

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Abstract

Complement, an enzyme cascade that forms part of the immune system, involves a multiple-step mechanism that is quite complex. Two initiation pathways can lead to activation of complement response; this work explores the kinetics of the so-called alternative pathway. Including sources of infection and an adjustable supply of the initial C3 protein in the pathway, a minimal model of the alternative pathway mechanism is constructed. Rate constants for the model are estimated from reported data, and the ordinary differential equations associated to the model are analyzed by numerical integration. This minimal model exhibits sustained oscillatory behavior. Such behavior is expected from a biological system with the feedback characteristics of the alternative pathway. With variation of a deactivation parameter, the oscillations undergo period doubling.

Keywords: Complement system; Immune response; Minimal model; Oscillatory behavior

1. Introduction

Complement, an immune system mechanism for cell destruction, is in many ways a well understood biological system [1,2]. Studies of human and murine complement proteins have yielded a wealth of structural and mechanistic information about this complex system of enzyme cascading processes [3,4]. Analysis of the kinetics of complement [5] has provided a general picture of the overall behavior of the system. However, the approximations required to make such an analysis mathematically feasible necessarily obscure much of the fine detail of the kinetic behavior of individual components of the system.

To study in detail the kinetics of single elements of complement as they behave under the influence of the entire system, this work employs numerical integration of mass action laws from a proposed minimal model derived from the accepted mechanism of the cascade. The mechanism is itself derived from experimental studies;

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simulations derived from this mechanism permit elucidation of kinetic behavior that may be masked by experimental conditions or the simplifications of previous kinetic analyses. Behavior expressed visually in the shape of the integration plots can then be translated to a mathematical description of the stability of the system.

In one sense, the motivation for this study of complement is the same as that for the volume of past and current experimental work on the system. Since complement forms an integral part of the human body's defense against disease, the more complete our understanding of its workings, the more ammunition we have in our ongoing battle against infectious diseases. Some diseases actually attack a portion of the complement system; for example, the Herpes Simplex I virus disables a pivotal complement protein [4], thus neutralizing that arm of the body's defense system. In case such as this, detailed information about complement may ultimately help provide a cure.

Beyond the importance of complement as part of the immune system, however, it merits close study simply as a member of a group of processes that proceed via enzyme cascade. The most famous member of this group is the blood clotting cascade, evidence of which sparked the development of cascade theory [6,7]. Studies of the kinetics of enzyme cascades [8] have, again, necessarily relied on gross approximations to make possible the analysis of large systems.

Like all enzyme cascades, complement exhibits a number of regulatory mechanisms for the purposes of discrimination and amplification. Unique to complement, though, is a feedback regulation system that results in the type of nonlinear dynamics associated with oscillators. Observed oscillations in such system as glycolysis [9] have received a great deal of attention; studies of this and other systems suggest a far-reaching importance of oscillation reactions in biological systems. Therefore, analysis of potential oscillatory behavior in complement may contribute to this field of study.

In section 2, we review the complement. The minimal model of the alternative pathway is discussed in section 3. The numerical results are presented in section 4 and followed by a discussion in section 5. Conclusion are presented in section 6.

2. The complement and enzyme cascades

In this section, we briefly review the kinetics of the enzyme cascades. Also, we review the role and mechanism of the complement system.

2.1 Kinetics of enzyme cascades

An enzyme cascade is defined [8] as an sequential array of enzymatic reactions in which the product of one reaction acts as the enzyme catalyst for the next reaction. The evolution of this type of mechanism, with its typically complex system of regulation, can be explained by the types of stimuli to which enzyme cascades respond. In addition to complement, well-documented cascades in blood plasma [10] include those responsible for blood clotting, breakdown of spontaneously formed blood clots (fibrinolytic cascade) and inflammatory response (kinin cascade). All of these processes have in common that a small fluctuation from normal must generate an immediate and strong response; the blood of a wounded person must clot, or that person will bleed to death. On the other hand, it is crucial that a response not be falsely generated; if a person's blood clots at a site where a wound has not occurred, death can result as well.

The general structure of a cascade accomplishes the first of these criteria, the amplification process. An enzyme molecule produced in one step can catalyze many subsequent steps, each of which can produce another molecule of enzyme, and so on. The all-important process of determining whether or not a stimulus is genuine occurs as a result of the complex regulation exhibited by cascading processes such as these.

Enzyme cascades generally fall into one of two categories: an open cascade, in which the product of each step is stable, or a damped cascade, in which the products are broken down as they appear. Figure 1 illustrates each of these two types of cascades. These diagrams are quite sim-

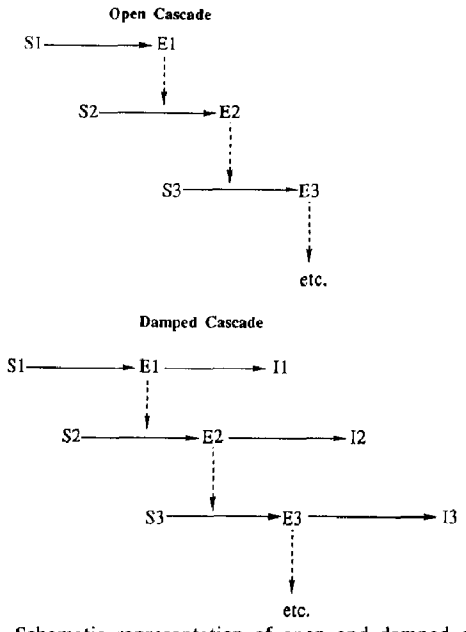

Fig. 1. Schematic representation of open and damped cascades. (S = substrate, E = enzyme, I = inactive form).

plified, obviously ignoring any regulatory effects. While complement can be considered a damped cascade, since its products are quickly inactivated unless they participate in the next step of the cascade, the interactions between components of complement are much more complex than the simple damped cascade model implies.

2.2 *The complement system*

When an infectious agent such as a virus or a bacterium enters the body, the immune system is capable of two main types of response. The first, cell-mediated immune response, involves reaction of specialized cells of the immune system with the infectious agent, or antigen, on the surfaces of infected host cells. The second type of immune response is a group of antigen-specific mechanisms, the antibody responses. Specialized proteins called immunoglobulins, each produced to bind to a specific antigen, circulate in the blood and permeate the body fluids. Once an antibody encounters its template antigen, it disables the antigen by one of two mechanisms. The antibody can simply inactivate the antigen by remaining bound to it, thus blocking the antigen's ability to bind to receptors on the target cells of the host. Alternatively, the antibody can serve as a marker for another agent to destroy the antigen, either in its free form or already attached to a host cell to form an immune complex. The two main destructive agents are phagocytic cells and the enzyme cascade known as complement.

Complement consists of twenty plasma proteins, including enzymes and binding proteins. The wider complement system includes surface receptors on both immune system and inflammatory cells, as well as regulatory membrane proteins on host cells to prevent autologous complement activation. Activation of complement takes place via two separate pathways which converge to initiate the cytolytic pathway of membrane attack. In each of the two activation pathways, an initial enzyme catalyzes the formation of the enzyme C3 convertase. Reaction of the C3 convertase with another enzyme in each of the pathways results in the formation of the enzyme C5 convertase, which in turn acts on the protein C5 to form the membrane attack complex (MAC). Formation of the MAC requires reaction of C5 with complement proteins C6–C9 to achieve polymerization of C9 within the target membrane. This polymerization creates transmembrane channels in the target cell, either the infectious agent itself or an infected host cell. The cell's contents then leak out through the channel, causing the death of the cell.

While the two activation pathways for the complement cascade follow the same general reaction scheme, they make use of different proteins and rely on distinct initiation mechanisms. Initiation of the classical, or antibody-dependent, pathway requires, as its name suggests, interaction with an antibody. The initial protein in this pathway is C1, which exists in two conformations. In the "closed" conformation, C1 remains incapable of autoactivation. In the "open" conformation, however, the catalytic sites of the protein become exposed. Autoactivation of C1 through its catalytic sites is normally prevented by binding with a protein called C1-In. If the C1 encounters an immune complex, though, binding of C1 to the

complex overcomes the controlling action of C1-In, and the classical pathway is initiated.

The second activation pathway of complement, called the alternative pathway, can operate without participation of an antibody. Instead, its activators include polysaccharides, fungi, bacteria, viruses, mammalian cells and aggregates of immunoglobulins [1]. These substances initiate the alternative pathway by affecting the microenvironment of the protein C3b, a subunit cleaved from the protein C3. Since cleavage of C3 occurs at all times by slow, spontaneous hydrolysis of a thioester in the protein, potential for activation of the alternative pathway sequence exists at all times. Normally, C3b binds preferentially to the inhibitor protein H, which halts the progress of the cascade. If the C3b "sees" the above mentioned signs of a foreign invasion, though, it binds preferentially to Factor B, which activates the complement response.

Both the classical and alternative pathways are characterized by a high degree of regulation. A complex system of inhibitors and activators prevents autologous complement attack and amplifies the effect of complement when an invasion triggers the immune response. Of particular note is a feedback regulation loop in the alternative pathway. In this case, the fragment C3b that is enzymatically cleaved from C3 by the initial C3 convertase forms a subunit of the initial C3 convertase itself. Therefore, when the microenvironment of deposited C3b particles corresponds to a foreign invasion, those particles will be activated rather than inhibited, allowing them to form more enzyme to cleave more C3b particles.

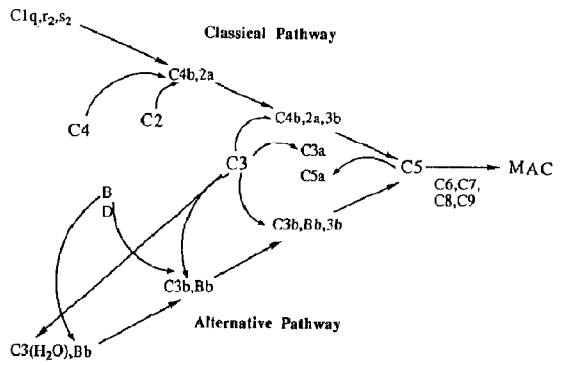

Fig. 2. Simplified diagram of complement mechanism.

A schematic diagram of complement is shown in Fig. 2. The two activation pathways require distinct forms of initiation; their regulation processes, while comparable in complexity, differ substantially in the details of their mechanisms. However, as the diagram indicates, the protein C3 plays a pivotal role in both pathways, and the end result of both pathways is the same process of MAC formation.

3. Alternative pathway model

As mentioned before, two features of the alternative pathway in the complement system make it unique: its antibody-independent initiation sequence and its feedback regulation mechanism. This feedback regulation, crucial in the amplification process of this pathway, results also in increased complexity of the kinetics of this portion of the system. Therefore, the initiation and feedback amplification mechanisms of the alternative pathway present a likely starting point for development of a minimal model of the complement.

3.1 Representation of the alternative pathway

The first step in the alternative pathway, which occurs at all times, is the slow, spontaneous hydrolysis of the thioester in C3 to form C3 (H_2O). Because water is in excess in the body, such a hydrolysis may be represented by the unimolecular reaction

$$A \xrightarrow{k_1} B2, \tag{1}$$

where A represents C3 and B2 C3(H_2O). Since the body is an open system, in which various substances are synthesized as they are broken down, we can the supply of C3 by a source. Hence, the first step becomes

$$\text{Source} \longrightarrow A \xrightarrow{k_1} B2. \tag{2}$$

Subsequent modifications in this model will explore the exact nature of this external source of C3; for now, though, a constant rate of synthesis is assumed.

Next in the alternative pathway comes the crucial activation step, in which C3(H_2O) reacts

with factors B and D to form the initial C3 convertase, $C3(H_2O)$,Bb. This initial convertase associates with a metal ion, usually magnesium (Mg^{2+}), so that the precise compound is $C3(H_2O)$,Bb(Mg). The formation of initial C3 convertase is a two-step process. First, $C3(H_2O)$ combines with Factor B in the presence of Mg to form $C3(H_2O)$,Bb(Mg). Factor D then cleaves B into two fragments: Ba, which dissociates from the enzyme complex, and Bb, which remains bound to form $C3(H_2O)$,Bb(Mg). In its full form, then, this step of the reaction is actually a two-step process,

$$C3(H_2O) + B \rightleftharpoons C3(H_2O),B \xrightarrow{D} C3(H_2O),Bb + Ba, \quad (3)$$

where B and D are Factors B and D, respectively.

Once the $C3(H_2O)$ is bound to Factor B, it has an overwhelming preference for binding to Factor D [3]. Therefore, since the reaction with Factor B is by far the rate limiting step in this activation process, the activation can be considered kinetically as a one-step process. Since the progress of the alternative pathway relies on increased production of hydrolyzed C3, for simplicity of this first model it is assumed that $C3(H_2O)$ in the initial C3 convertase or, as detailed below, C3b in the C3 convertase of the activated pathway, is the limiting concentration in this step. Therefore, Factors B and D are currently eliminated from the model. The final result of this contraction is a one-step, unimolecular process,

$$B2 \underset{k_{a_1}^-}{\overset{k_{a_1}^+}{\rightleftharpoons}} E1, \quad (4)$$

where E1 stands for the first enzyme produced in the pathway, the initial C3 convertase $C3(H_2O)$, Bb(Mg); B_2 is the $C3(H_2O)$ from eq. (2).

Once the initial C3 convertase is formed, it acts on C3, cleaving it to form the reactive particle C3b. With C3 written as A from eq. (2), and the particle C3b as Ab, this step can be written as

$$A \xrightarrow{E_1} Ab. \quad (5)$$

Expanded according to the Michaelis–Menten model, with C1 as the enzyme–substrate complex, eq. (5) becomes

$$A + E1 \underset{k_{E_1}^-}{\overset{k_{E_1}^+}{\rightleftharpoons}} C1 \xrightarrow{k_{E_1}} E1 + Ab. \quad (6)$$

Once C3b is formed, it is deposited on surrounding particles in the plasma. When complement has been activated — that is, when the host body has been invaded by some infectious agent — some of the C3b falls on either on foreign bodies themselves or on infected cells of the host body. When C3b recognizes an infected microenvironment, it exhibits a strong binding preference for Factor B, the same protein that helps activate the initial C3 convertase. The same process of activation takes place, with Factor D cleaving part of the bound Factor B, to form the C3 convertase of the activated alternative pathway, C3b,Bb. This step is represents as

$$C3b + B \rightleftharpoons C3b,B \xrightarrow{D} C3b,Bb + Ba. \quad (7)$$

Comparison of eq. (7) with eq. (3) shows that C3b acts just like $C3(H_2O)$ in this case. Furthermore, the C3 convertase formed from C3b performs the same function as the initial C3 convertase formed from $C3(H_2O)$. This allows a simplification of the model; instead of adding a separate step for the formation of C3 convertase from C3b, the feedback of C3b is shown directly by making its activation analogous to the formation of more $C3(H_2O)$. Schematically, with Ab as C3b and B2 as $C3(H_2O)$, this step is

$$Ab \xrightarrow{k_2} B2. \quad (8)$$

Thus, this portion of the system is contracted to include one enzyme instead to two. The rate constant k_2 can be considered as a recognition parameter, representing the relative speed with which deposited C3b particles recognize an activating microenvironment and respond to it.

The only remaining aspect of this portion of the alternative pathway is the deactivation that is responsible for inhibiting alternative pathway activity when no infection is present, thereby helping to prevent autologous complement attack. The main deactivators in this mechanism are

Factors H and I, which bind cooperatively to both C3(H_2O) and C3b. The mechanism for deactivation is much like the activation mechanism involving Factors B and D. First, H binds reversibly to the target molecule, and then the protease I attacks this complex. Because the serum concentration of H is relatively large compared to the dissociation constant of C3b,H (and presumably the dissociation constant for the C3(H_2O) analog), and because I is a particularly efficient protease [3], in the absence of any activating agent, the deactivation of these species is virtually an irreversible process. An analogy can be made with the contraction made in eq. (4); here, though, it is as if the dissociation rate (represented by the constant k_{a1}^- in the activation process) were so low in comparison with the association (forward) rate that the entire process goes in one direction. Therefore, in our model the deactivation step is contracted to a unimolecular, irreversible process. Equations (9) and (10) are the last two steps in this first part of the model, showing the deactivation of C3b and C3(H_2O), respectively:

$$B2 \xrightarrow{k_{d1}} I_1, \tag{9}$$

$$Ab \xleftarrow{k_{d1}} I_2. \tag{10}$$

where I_1 and I_2 refer to the inactive forms in each step. Since the same deactivation mechanism applies to both species, the approximation is made that the rate constants for both deactivations are the same.

An overview of this basic model and its correspondence to the actual alternative pathway mechanism is presented in Figs. 3 and 4.

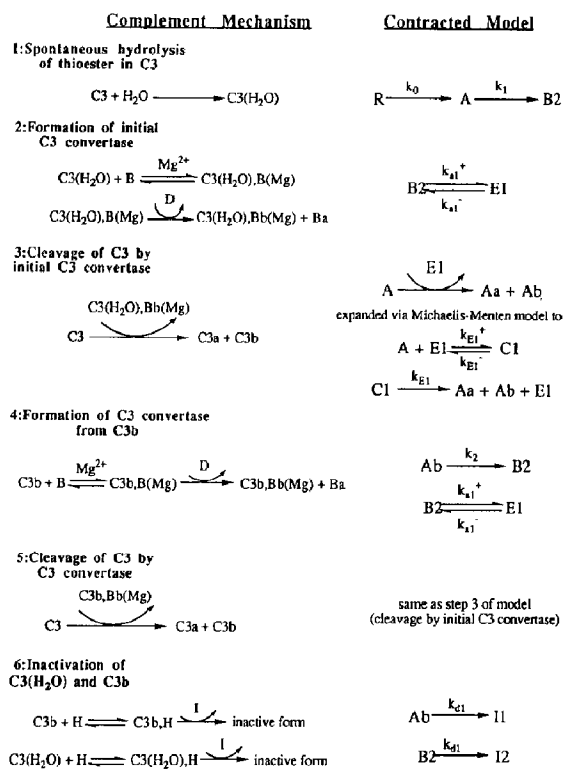

Fig. 3. Comparison of model to actual complement mechanism.

3.2 Assignment of rate constants

Some of the rate constants in this basic complement model have determined values; others obey certain numerical relationships to each other. The rate constants for which no values are available serve as free parameters to the extent that they may be varied within physiological bounds.

The reported half-life of the C3 thioester is 231 hours at 37°C [3]. The resulting rate constant, k_1, is determined from the rate law for the first-order hydrolysis step, which yields the relationship

$$\ln\left\{\frac{A(t_{1/2})}{A(t_0)}\right\} = -k_1\{t_{1/2} - t_0\} \tag{11}$$

or $k_1 = 3.00 \times 10^{-3}\ h^{-1}$.

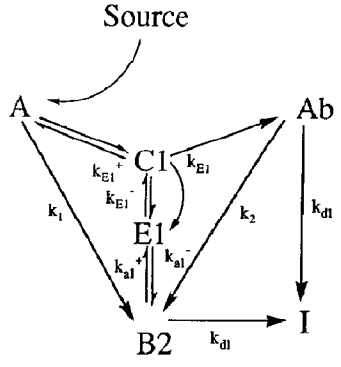

Fig. 4. Schematic diagram of basic alternative pathway model.

The specific enzymatic activity (k_{cat}/K_m) of C3 convertase is reported [3] as $1.6 \times 3.1 \times 10^5$ M^{-1} s^{-1} This dictates a quantitative relationship between the rate constants for the equilibrium process of enzyme–substrate complex formation, k_{E1}^+ and k_{E1}^-, and the rate constant for product formation, k_{E1}, according to the following relationship:

$$\frac{k_{cat}}{K_m} = \frac{k_{E1}k_{E1}^+}{k_{E1}^-}. \tag{12}$$

Using typical values for enzymatic reactions, the values chosen are $k_{E1}^+ = 3.6 \times 10^{12}$ M^{-1} h^{-1}, $k_{E1}^- = 3.6 \times 10^{10}$ h^{-1}, and $k_{E1} = 7.2 \times 10^6$ h^{-1}.

A decay-dissociation half-time of 90 s is reported for C3 convertase [3]. This value is converted to the rate constant for the reverse of the equilibrium enzyme activation step, $k_{a1}^- = 36$ h^{-1}. A value for the forward process of this equilibrium can be estimated from general kinetic values for enzymatic reactions as approximately one-third the reverse value, or $k_{a1}^+ = 10.8$ h^{-1}.

The deactivation rate constant for C3b and C3(H_2O), k_{d1}, is determined from the data for the protease Factor I. The Michaelis–Menten constant K_m for I is reported as 2.5×10^{-7} M [3]. The resulting approximation for the unimolecular rate constant is $k_{d1} = 3.6 \times 10^6$ h^{-1}.

The free parameters that remain, then, are the external source of C3 into the system and the parameter for the activating microenvironment of deposited C3b particles, k_2. It is also important to note that the parameter k_{a1}^+, while assigned a numerical value on the basis of the given value for k_{a1}^-, still allows for a measure of variability.

With a constant rate of synthesis, this basic model shows damped oscillations. Thus we have the option of modifying the model and look for sustained oscillation or analyzing, at a biochemical level, the source of C3 in the complement.

The first two models of oscillatory systems ever developed provide an example of the first type of modification. Alfred Lotka published in 1910 a model of an open system that exhibits damped oscillations [11] and in 1920 a model of a system exhibiting sustained oscillations [12]. The difference between the 1910 and the 1920 versions of this model is an autocatalytic formation of the first species in the mechanism, resulting in a shift from damped to sustained oscillations. The analogous change in our first complement model would be an autocatalytic production step for C3, or A. In fact, this change does result in sustained oscillations [13]. However, the direct autocatalytic formation of C3 has no physiological analog, since autocatalytic formation prior to recognition of actual infection would lead to unnecessary buildup of C3 in the body. Thus, in this way we are virtually forcing oscillations on the system through an artificial autocatalytic step.

3.3 Sources of C3

The next step, then, is to expand that model in such a way that it more closely reflects the actual complement mechanism. Presumably, if sustained oscillatory behavior exists anywhere in this system, increasing the complexity of the model will bring the system close to the region of that behavior, since increased complexity leads to more interdependent relationships among the species of the system.

The site chosen for expansion was the external source of C3 (A). As explained previously, in the case of the basic model we have approximated the external source of C3 as a low-level, constant supply of that protein. While this approximation does reflect the body's ability to manufacture additional C3, it is a rather simplistic version of how the body maintains its non-equilibrium state. In this expansion of that model, two different sources of C3 are taken into account: a constant, adjustable pump that maintains a standard concentration of C3 and an infection-activated response that stimulates increased production of C3 when the alternative pathway is activated. Constant supply of C3 is represented in the minimal model by

$$(A_o - A) \xrightarrow{k_o} A, \tag{13}$$

which is a typical representation used in continuous-flow stirred-tank reactions (CSTR). The term $(A_o - A)$ represents an adjustable source of A.

Since most C3 is manufactured in the liver, this term can be seen to represent the production of C3. In this model, those cells responsible for C3 synthesis would somehow receive information about the body's concentration of C3. They would then adjust their rate of synthesis according to that information. For example, when the level of C3 in the blood is exactly the standard level ($A = A_0$), the term ($A_0 - A$) would be zero, and production of C3 would temporarily cease. If the concentration of C3 were close to zero, then production would reach a maximum rate of $k_0 A_0$. In this adjustable source of C3, the rate constant k_0 acts as a measure of how fast the liver responds to changing concentrations of C3 in the blood.

Stimulated production of C3 in response to activation of the alternative pathway corresponds to experimental data showing that Interleukin-1 (IL-1), a protein produced at the site of infection, causes substantial increases in the rate of production of C3 [14]. Because our model does not take into account any immune system proteins outside the complement pathway, an approximation is needed to link this response to complement. That approximation substitutes C3b (Ab), the latest component of the alternative pathway studied in our minimal model, for IL-1. The significance of this substitution is that C3b is only present in high levels when infection is recognized. Therefore, inclusion of C3b in the stimulatory response introduces the same kind of "marker" for infection that IL-1 does. The second component of the stimulatory response is the actual infection itself, because it seems accurate to have the stimulatory response grow as the actual concentration of infectious agents in the body increases. In addition, requiring the presence of actual infectious agents provides another check on accidental inflammatory response, which is an important aspect of the alternative pathway activation.

Stimulatory activity comes from a step involving both the infection, Z, and the protein marker, Ab:

$$Z + Ab \xrightarrow{k_{0A}} A + Ab, \qquad (14)$$

where Z, represents the infectious presence in the body. Since Ab serves only as a recognition factor, it is not consumed in the reaction. In this model, Z is used up in the production of more A. Since the pathway is not yet completed to MAC assembly and cell death, removing the infectious agent in this manner provides an intermediate means of closing off the pathway.

Infection in the minimal model is represented by a slow, constant introduction of infection, followed by a reproductive step. The reproduction is modeled as a simple fission, corresponding to bacterial reproduction. Together, these two steps result in the equation

$$\text{Source} \xrightarrow{k_{\text{infec}}} Z \xrightarrow{k_{\text{rep}}} 2Z. \qquad (15)$$

A complete set of reactions for the minimal model is shown in Fig. 5. Schematic diagram of the minimal model is presented in Fig. 6.

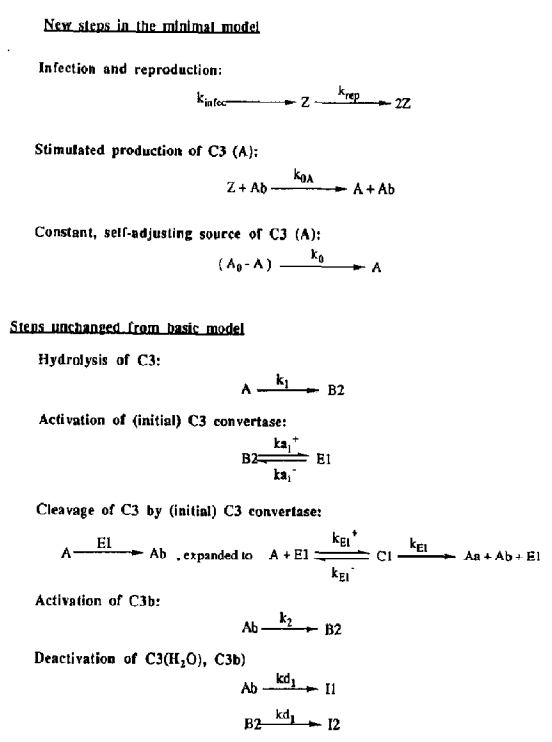

Fig. 5. Mechanism of the minimal model of the alternative pathway of the complement.

4. Numerical results

The mass action laws for the minimal model are as follows:

$$\frac{dZ}{dt} = k_{rep}Z + k_{infec} - k_{oA}Z\,Ab \tag{16}$$

$$\frac{dA}{dt} = k_o(A_o - A) + k_{oA}Z\,Ab + k_{E1}^{-}C1 - k_1 A - k_{E1}^{+}A\,E1 \tag{17}$$

$$\frac{dB2}{dt} = k_1 A + k_2 Ab + k_{a1}^{-}E1 - k_{a1}^{+}B2 - k_{d1}B2 \tag{18}$$

$$\frac{dC1}{dt} = k_{E1}^{+}A\,E1 - k_{E1}^{-}C1 - k_{E1}C1 \tag{19}$$

$$\frac{dE1}{dt} = k_{E1}^{-}C1 + k_{E1}C1 + k_{a1}^{+}B2 - k_{a1}^{-}E1 - k_{E1}^{+}A\,E1 \tag{20}$$

$$\frac{dAb}{dt} = k_{E1}C1 - k_2 Ab - k_{d1}Ab \tag{21}$$

Notice that this set of differential equations has only two bimolecular steps. Consequently, the highest nonlinearity is of order two. Moreover, the only autocatalytic step is of order one.

This set of ordinary differential equations have been integrated using PLOD [15] implementation of the Gear [16] algorithm with accuracies ranging between 10^{-10} to to 10^{-12}. The parameters used for the minimal model are given in Table 1. The only new parameter with a literature value is A_o, the standard plasma concentration of C3, given as 7.2×10^{-6} M [2].

Using this set of parameter values, integration of eqs. (16)–(21) results in sustained oscillations in the concentrations of all species in the system. Figures 7a–7f shows a series of oscillations of this nature.

Some variation of parameter values was used to explore this oscillatory region. The enzyme activation parameter k_{a1}^{+}, which was characterized as a particularly sensitive variant in the damped oscillations of the first model, proved to affect mostly the shape of the oscillatory peaks rather than to change their actual existence. An example of the effect of k_{a1}^{+} on the shape of oscillations is given in Figs. 8 and 9, which show enlargements of two peaks at different values of k_{a1}^{+}. When k_{a1}^{+} is raised by three orders of magnitude, the peaks develop a much narrower spike at the top than is seen for the lower value of 10.8.

Variation in the value of k_{rep} showed changes in the shape, rather than in the existence, of the observed oscillations, as well. A typical phase plane diagram of the species Z and Ab, chosen for their role as "endpoints" of the model, is shown in Fig. 10. The shape of the peaks obtained corresponds to a type of behavior known as relaxation oscillations. In this case, their shape and order to onset can best be interpreted as a

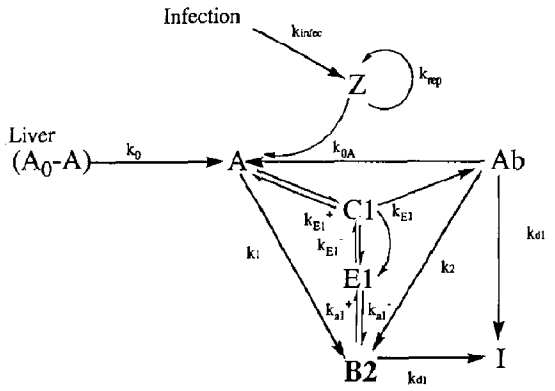

Fig. 6. Schematic diagram of the minimal model of the alternative pathway.

Table 1

Assigned rate constant

Rate constant	Assigned value
From basic model	
k_1	3.0×10^{-3} h^{-1}
k_{a1}^{+}	10.8 h^{-1}
k_{a1}^{-}	36 h^{-1}
k_{E1}^{+}	3.6×10^{12} M^{-1} h^{-1}
k_{E1}^{-}	3.6×10^{10} h^{-1}
k_{E1}	7.2×10^{6} h^{-1}
k_{d_1}	3.6×10^{6} h^{-1}
Added in minimal model	
k_o	1.0×10^{-1} h^{-1}
k_{oA}	1.0×10^{13} h^{-1}
k_{infec}	1.0×10^{-6} h^{-1}
k_{rep}	1.0

gradual buildup of Z, the infectious presence, which eventually triggers a sharp increase in the concentrations of the cascade species. The sudden increase in concentration of these species results in a sharp decrease in the concentration of Z, and the process then repeats itself. The onset of oscillations occurs after approximately a ten-hour buildup of the concentration of infectious agents in the body.

Characterization of this system with respect to

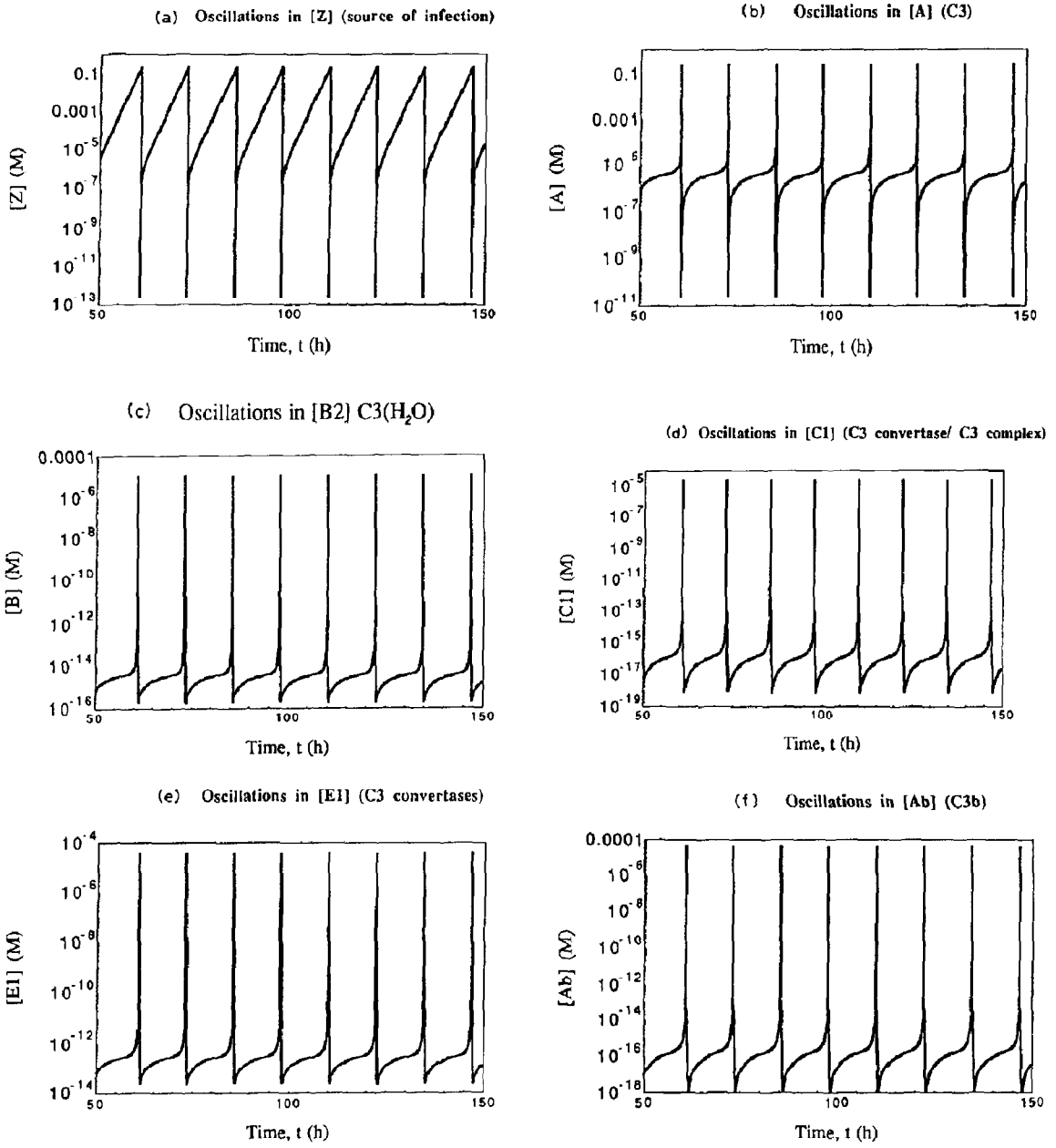

Fig. 7. Oscillation found for the parameters in Table 1.

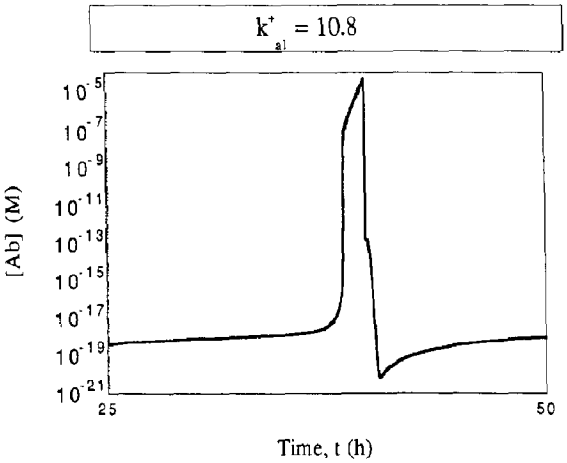

Fig. 8. Peak shape of [Ab] for $k_{a1}^{+} = 10.8$.

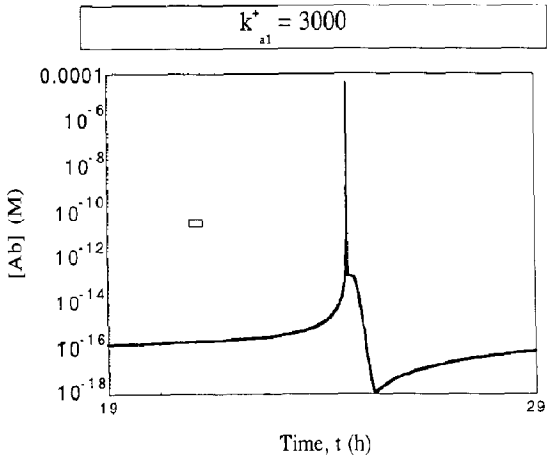

Fig. 9. Peak shape of [Ab] for $k_{a1}^{+} = 3000$.

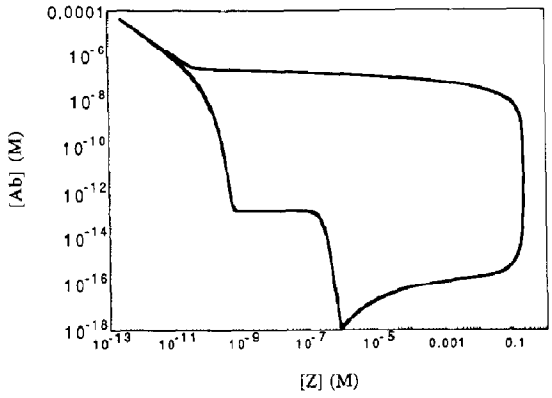

Fig. 10. Phase plane for sustained oscillations in the minimal model. Parameter values as in Fig. 7.

various parameters led to the discovery of the onset of period doubling. The parameter varied in this case is k_{d1}, the rate constant for deactivation of C3(H_2O) and C3b. When the original value of k_{d1}, $3.6 \times 10^6 h^{-1}$, is lowered by four orders of magnitude, the peaks of the original oscillations are split into two peaks, a smaller one followed by a larger one. This phenomenon is known as period doubling. Two separate regions of period doubling occur, distinguished by the shapes and relative magnitudes of their respective peak pairs. Figure 11 and 12 show representative plots from the two regions of period doubling, at $k_{d1} = 300\ h^{-1}$ and $330\ h^{-1}$, respectively. A typical phase plane diagram, again for Z and Ab, is shown in Fig. 13. The two separate loops correspond to the two different types of peaks in this region. The apparent crossing of the loops is not an actual crossing, but a result of the projection of a six-dimensional system onto two dimensions. Comparison of this diagram to the phase plane diagram of the single-peak oscillations (Fig. 10) shows that the loop for period-doubling more close approaches the usual smooth shapes traditionally seen for oscillatory systems.

As we mentioned earlier, period doubling appeared for values of k_{d1} four order to magnitude smaller than the estimated value from experimental data. Whether this value are physically plausible or not is the subject of a different study. Also the purpose of this work is to present a minimal model of the alternative pathway of the complement with physical parameters estimated from experimental data. Nevertheless, a further and more freely analysis in parameter space has been discussed elsewhere [17].

5. Discussion

The sustained oscillations observed in our minimal model of the alternative pathway have implications for both the physiological and mathematical aspects of this work. The physiological significance of these results relies on the derivation of the model from an actual biological mechanism and on the plausibility of the outcome. The mathematical significance lies simultaneously in the

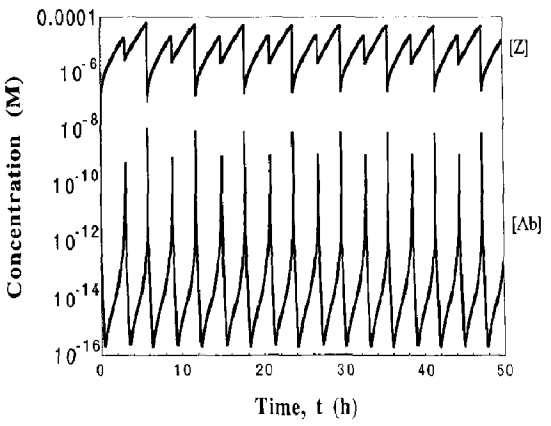

Fig. 11. Period two oscillations for $k_{d1}^{+} = 300$.

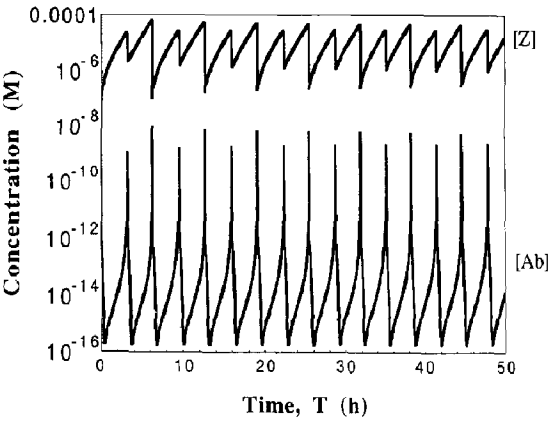

Fig. 12. Period two oscillations for $k_{d1}^{+} = 330$.

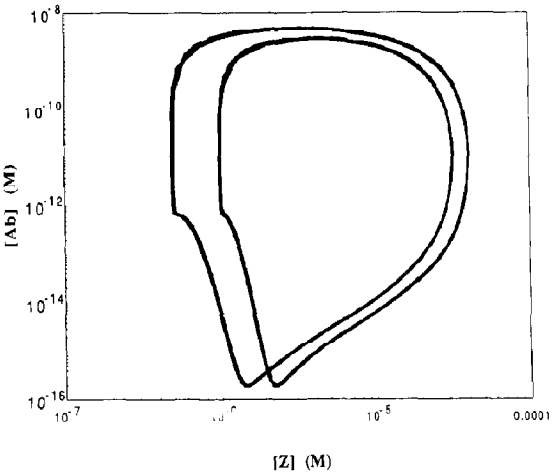

Fig. 13. Phase plane for period two oscillations for $k_{d1}^{+} = 300$.

simplicity of the model and in the complexity of its resulting dynamics.

5.1 Physiological accuracy of results

What separates this model from so much of the work that has been done to date on biological mechanisms is the degree of its correspondence to the actual mechanism. In many cases, potentially oscillating mechanisms are constructed and their relation to actual mechanisms derived later. The fact that this model was derived with concern first for its validity as an approximation of the real complement system, and that oscillations resulted, makes those oscillations physiologically more plausible than if the process had been reversed.

It is true that in the minimal model, several approximations are made in the interest of simplifying characterization of the model's kinetic behavior. However, it is our belief that these approximations still reflect accurately the most important characteristics of the alternative pathway, namely the recognition-dependent activation of C3b and the positive feedback loop involving that species. Indeed, the simplifying approximations should, if anything, make the kinetic behavior of the model simpler than if those approximations were eliminated. A more complex model should show more complex behavior. Therefore, the oscillatory behavior of the simplified model is not expected to disappear with increased complexity of the model; if anything, that behavior should become richer.

A useful example of this parallel trend in complexity can be found in the expansion between the basic and minimal. The change from the basic to the minimal model was prompted by the conclusion that the behavior of the first model was fully characterized within physiological bounds of the parameters. The exact nature of the change, though, was a response to that area of the model which seemed the least accurate in the first approximation — the source of C3. The CSTR mechanism introduced as a constant source is a common method for modeling an open system with response-adjusted rates of input and output of reactants, and seems the natural next

step in modeling the input of C3. The stimulatory response seems also a natural addition to the model, based on the idea that, in the case of infection, the normal routes of C3 production may not be enough to keep up with the increased demands of the alternative pathway. That such a mechanism has been studied with respect to IL-1 further supports this idea. The addition of an infection and reproduction step was an obvious one as well, since the infectious agent serves as both the starting point for the activation of the alternative pathway and, in the event of its total removal from the body, as the endpoint of that reaction. Thus, all three of these additions to the first model accomplish the goal of increasing the accuracy of the model. The increased complexity of the kinetic behavior of the model — from damped to sustained oscillations — results from the increased complexity and accuracy of the model. Thus, the oscillations obtained from our minimal model can be considered superior to those obtained by virtually forcing oscillations on the system through an artificial autocatalytic step.

Another feature of our model which contributes to its validity is the availability and use of actual rate constant values for the steps of the alternative pathway mechanism. While some of the approximations make direct translation of these constants impossible, many of the steps allow direct use of the literature values for these constants. Even in those steps for which no constants are available — particularly the steps added in the expansion of the model — the parameter values used fall well within the range of acceptable constants for biological systems. The fact that these constants were assigned on the basis of the model and not on the basis of the behavior desired for the model makes the results that much more significant.

One check on the accuracy of such a model is a comparison of the theoretical results with experimental ones. For this system, no values for *in vivo* concentrations of alternative pathway species as a function of time are available. However, making sure that the theoretical results fall at least within physiologically reasonable ranges serves the same purpose to a lesser degree. In the minimal model, however, the maxima of the oscillations fall mainly within the micromolar (10^{-6}) to millimolar (10^{-3}) range. These concentrations are both expected and detectable. The fact that the minimal model exhibits more reasonable concentrations seems like another affirmation of the increase accuracy as compared with the basic model.

One potential glitch in the concentrations in the minimal model is that the oscillations in Z, the infectious agent, can reach values up to 1 *M*. Under a normal interpretation, this concentration is probably much too high to be physiologically acceptable. An explanation, though, may be provided by the fact that the model reflects local as well as overall concentrations. Once C3b is deposited on particles in the body, the pathway operates largely via a membrane-bound mechanism. In addition, the stimulatory response of IL-1 corresponds more to a local site of infection than to an overall response in the body. This unnaturally high value for [Z], then, may reflect more of a local than an overall concentration, in which case it would be acceptable.

As a final interpretation of the physiological accuracy of the results of the minimal model, the question of whether or not oscillations should be expected in the first place seems a valid one. As mentioned in the first discussions of the alternative pathway, the mere existence of a feedback loop is enough at least to suggest that oscillations are present somewhere in the system. The fact that this is a biological system makes the prospects even likelier, since other biological systems are known to oscillate. It is interesting to note that the oscillations observed are on the time scale of hours, in some cases reaching almost a circadian cycle, which suggests further compatibility with other biological processes [18].

5.2 Mathematical implications of results

Considering that the autocatalytic step is of first order, that the highest nonlinearity is quadratic, and that most of the steps in the mechanism are first order decays, the type of oscillations observed in numerical integration of the minimal model are rather unusual. The first concern in analyzing such behavior is to ensure

that it is not just a numerical phenomenon-that is, a deformation of some sort in the shape of oscillations because of the integrator's inability to cope with the "stiffness" of the system (vast disparities between the orders of magnitude of parameters in the model). Such phenomena can result even when using the Gear algorithm, which is specifically designed to work with stiff systems This possibility is ruled out in our case by increasing the integration accuracy until no change is seen in the shape of the oscillations. All plots included here are the result of integration at 10^{-10} accuracy, but the exact same results were obtained over the range beginning at accuracy of 10^{-8} to 10^{-12}.

Once the possibility of numerical inaccuracy is eliminated, the model itself can be considered to be the source of the unusually shaped phase plane diagram shown in Fig. 10. Typical diagrams of this type, plotting one parameter of an oscillating system versus another, give smooth, curved loops. In the case of a pure sine wave oscillation, the phase plane diagram is a circle; as the oscillations deviate from this standard, the circle becomes somewhat distorted. However, the sharp changes from vertical to horizontal exhibited in our phase plane diagram are quite unusual. The resulting peak structure is unusual as well, though the differences show up more clearly in the phase plane diagram. These peaks are not of the sawtooth variety, but more of the relaxation variety, as previously mentioned, and they exhibit a degree of structure.

The mathematical significance of the period doubling effects described in previous section is perhaps even greater. Period doubling is the classical route to chaotic behavior. The next step to chaos from the period doubling already observed would be yet another period doubling, to period four oscillations. Beyond this doubling, the next change would be to period eight, with chaos occurring either at or directly after this third doubling region.

A prototype for chaos in biological systems [19,20], and specifically in the body, is the chaotic behavior of brain waves in humans. However, during an epileptic seizure, the brain waves of the seizure victim turn periodic [21]. This instance of chaos as a sign of health is a powerful analogy, since the potentially chaotic behavior we observe comes as a result of modeling a malfunction in complement, i.e. a lowered activity of Factors I and/or H.

Both the oscillating and period doubling behavior of this model share the mathematical significance of occurring within a relatively simple model. Complex models have been constructed in the past with the expressed purpose of simulating chaotic behavior. Those models often include a number of nonlinear steps, sometimes even the chemical equivalent of trimolecular collisions, which are highly unlikely in physical terms. Our model, however, contains very few nonlinear steps. Only the enzymatic cleavage of C3 and the stimulation of increased C3 production involve bimolecular steps; the infection reproduction step is the only strictly autocatalytic step, and it is not an approximation at all, but a classical equation for bacterial reproduction. The remainder of the steps are unimolecular ones. Therefore, our model is both less complicated and more physically based than models exhibiting correspondingly complex kinetic behavior. This mathematical fact provides yet another motivation for the simplifications used in constructing our original model: not only to they allow for easier analysis, but they also provide data about what components are necessary for the behavior observed without confusing the issue with extra mechanistic details.

6. Conclusions

Our minimal model of the alternative pathway of complement represents the first modeling work of this kind performed on this system. That a model designed directly from an actual biological mechanism, and described by experimentally obtained parameters, exhibits oscillatory behavior strongly suggests that such behavior forms a legitimate part of this system. Because of its relative shortage of complex individual steps, this model represents one of the simplest models for which such behavior has been observed. Considering the relative simplicity of the model, which in-

cludes only two bimolecular steps and a first order autocatalytic step, the types of oscillations found in the system of differential equations possess a unique shape, which makes them interesting even from a purely mathematical point of view. The appearance of period doubling opens up the possibility of more complex dynamics in the system, a possibility that should grow stronger as the system's mechanism complexity is increased in future studies. Ultimately, we hope that studies such as this will lead to experimental research to verify the existence of the time behavior our model predicts.

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