A Comparison of Variant Theories of Intact Biochemical Systems. I. Enzyme–Enzyme Interactions and Biochemical Systems Theory*

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ABSTRACT

The need for a well-structured theory of intact biochemical systems becomes increasingly evident as one attempts to integrate the vast knowledge of individual molecular constituents, which has been expanding for several decades. In recent years, several apparently different approaches to the development of such a theory have been proposed. Unfortunately, the resulting theories have not been distinguished from each other, and this has led to considerable confusion with numerous duplications and rediscoveries. Detailed comparisons and critical tests of alternative theories are badly needed to reverse these unfortunate developments. In this paper we (1) characterize a specific system involving enzyme–enzyme interactions for reference in comparing alternative theories, and (2) analyze the reference system by applying the explicit S-system variant within biochemical systems theory (BST), which represents a fundamental framework based upon the power-law formalism and includes several variants. The results provide the first complete and rigorous numerical analysis within the power-law formalism of a specific biochemical system and further evidence for the accuracy of the explicit S-system variant within BST. This theory is shown to represent enzyme–enzyme interactions in a systematically structured fashion that facilitates analysis of complex biochemical systems in which these interactions play a prominent role. This representation also captures the essential character of the underlying nonlinear processes over a wide range of variation (on average 20-fold) in the independent variables of the system. In the companion paper in this issue the same reference system is analyzed by other variants within BST as well as by two additional theories within the same power-law formalism—flux-oriented and metabolic control theories. The results show how all these theories are related to one another.

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1. INTRODUCTION

An appropriate language or formalism with which to analyze complex biochemical systems has been sought for more than two decades. The necessity for such a formalism results from the large number of interacting components in biochemical systems and the complex nonlinear character of these interactions. (For a brief review see [42].) Two well-known formalisms that frequently have been used for analysis of biochemical systems are the linear formalism (e.g., [1], [14], [29]) and the Michaelis–Menten formalism[1] (e.g., [11], [12], [72]).

The linear formalism is among the best understood and best developed mathematical structures. A linearized description of a biochemical system can be efficiently treated mathematically in many different ways, even when there are hundreds of system components. It is a general symbolic formalism guaranteed to be valid over at least some restricted range of the concentration variables. However, biochemical systems are often highly nonlinear, and therefore the linear formalism, which cannot represent known nonlinear properties of biochemical systems, is inappropriate.

The Michaelis–Menten formalism, on the other hand, approximates many individual reactions reasonably well in vitro and presumably in vivo. Descriptions in this formalism are readily utilized as long as only one enzyme or a system of very few enzymes is being studied. However, under physiological conditions, each enzyme is not isolated but interacts with other enzymes and structures embedded in an intricate network of reactions. The Michaelis–Menten formalism does not produce a systematically structured formalism appropriate for analysis of such complex systems. The central assumptions of this formalism restrict its application to systems with independent rates that are linear functions of enzyme levels and activities. The resulting formalism leads to ad hoc mathematical descriptions that are not easy to study analytically.

The first formalism to differ significantly from these two is the power-law formalism developed in the late 1960s [32–37]. This formalism represents the interactions of a system in a structured fashion that greatly facilitates analysis, and yet it retains the essential character of the underlying nonlinear

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[1]By the Michaelis–Menten formalism we do not mean just the original Michaelis–Menten assumptions, derivation, and specific rate law [25] but also the broader spectrum of subsequent developments in enzyme kinetics that nonetheless share key assumptions and empirical methodology (e.g., [26], [23], [4]). For example, the assumption that there are no interactions between the various enzyme forms in a mechanism or between forms of other enzymes yields steady-state equations that are linear in the concentration of enzyme forms [4]. Solution of these equations produces rate laws that are linearly related to the concentration of total enzyme [32, 71].

processes [37, 40, 41]. (For further discussion of the strengths and weak-nesses of this formalism, and comparisons with the linear formalism and the Michaelis–Menten formalism, see [37], [40], [49], and [62].) This approach was combined by Savageau with the well-established network theory origi-nally developed by Bode and others [2, 8, 24, 58], and the result provided the basis for a new theory of intact biochemical systems [35–37, 40, 50], which is now called biochemical systems theory (BST). A particular variant within BST has been emphasized because of its greater structural clarity, analytical power, and accuracy [33, 37, 40, 42, 49, 50, 62]. This is called the S-system variant because it involves a mathematical representation, the S-system, developed specifically for synergistic and saturable systems. It has been successfully applied to a large number of biochemical systems, and specific predictions of the theory have been confirmed by independent laboratories (for a brief review, see [43]).

A second new formalism, believed by some to provide a theory that is generally applicable and independent of others, was presented in the mid-1970s [15, 16, 21]. The basic principles of this approach, which has been called metabolic control theory (MCT), are provided by special "summation" and "connectivity" relationships. However, the advocates of this second formalism have not provided any evidence to document that it differs from the power-law formalism. Yet a third new formalism, also considered by some to provide a generally applicable and independent theory, was pro-posed in the late 1970s [5–7]. This formalism has been referred to as a flux-oriented approach, so the theory provided by this approach will be referred to here as flux-oriented theory (FOT). The advocates of this third formalism also have not documented that it differs fundamentally from the others.

The introduction of these alternatives without distinguishing them from existing theories has led to considerable confusion in the field, to numerous duplications and rediscoveries, and to needless proliferation of notation. Progress toward understanding intact biochemical systems is in danger of becoming fragmented into a number of seemingly unrelated approaches. In an effort to reverse this unfortunate development and to begin establishing the relatedness of these approaches, we previously have given a general comparison of MCT with BST [49, 50, 62].[2] The results demonstrated (1)

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[2] We have chosen to make comparisons of BST with MCT rather than with the approach of Crabtree and Newsholme, at least initially, because MCT uses an implicit approach that makes its relatedness to other approaches more difficult to discern. Once the confusion resulting from implicit rather than explicit methodology is dispelled, the related-ness of all three approaches will become more readily apparent. We shall consider the approach of Crabtree and Newsholme in part II of this series of papers [54].

that both BST and MCT are based on the underlying power-law formalism; (2) that all results that can be obtained in principle with MCT can be obtained in principle with BST, while the converse is not true; and (3) that the variant of the power-law formalism implicit in MCT is less accurate than the S-system variant represented within BST. As a consequence, MCT is a special case within the larger conceptual and analytical framework of BST.

The implications of these general comparisons based on the logical content of alternative theories become clearer when one examines the results of specific experimental applications. Previous applications of MCT (summarized in [22]) have not produced any result that distinguishes this theory from BST. Moreover, all of these applications are incomplete. Only changes in certain component parameters have been attempted, and the specificity of these is difficult to document quantitatively. Conversely, only certain systemic responses have been examined. In no case has a complete and rigorous analysis of an intact system been presented. This is understandable given the technical limitations in any experimental approach. Hence, this is not meant as a criticism of the experimental work, but as a note of caution regarding the acceptance of claims made for a theory that has not been critically tested.

To move forward with a program of clarification, and to make evident the consequences of fundamental similarities and differences [49, 50, 62], the focus must shift to the results of concrete applications and critical experiments that *discriminate* between alternative theories. If FOT or MCT has an advantage not possessed by BST, then one should be able to demonstrate this by the design and execution of a critical experiment. Conversely, if FOT and MCT are simply a subset of BST, then one should be able to propose a critical experiment for which BST correctly predicts the outcome while the others do not.

In this series of two papers we present such a critical comparison involving a mechanism with enzyme–enzyme interactions. The system selected is ideal. Its characteristics are well defined theoretically, and it can be used to generate all the empirical data needed for testing a complete and rigorous analysis of the system. The principal objectives of this first paper are (1) to present and characterize the system that will become the reference for comparisons among the various theories of intact biochemical systems, and (2) to perform a complete analysis of the reference system using the S-system variant within BST.

The results are important in their own right. They provide the first analysis of enzyme–enzyme interactions within the class of theories based on the power-law formalism, the first example of a complete and rigorous analysis of a specific biochemical system, and additional evidence for accuracy of the S-system representation within BST.

In the second paper [54] we analyze the same reference system by two additional variants within BST and by two other theories based on the power-law formalism—FOT and MCT. The results allow one to discriminate among the variants and to show how all the theories are related.

2. ENZYME–ENZYME INTERACTIONS

There is now abundant evidence for the existence of interactions between enzymes and between enzymes and structural elements within the cell. These interactions lead to the formation of multienzyme complexes as in the well-known cases of the pyruvate dehydrogenase and fatty acid synthetase complexes [30]. Two types of rationales have been advanced for such spatial organization in vivo: catalytic efficiency and regulatory effectiveness.

When enzymes carry out a sequence of reactions, complexes among consecutive enzymes can promote the catalytic efficiency of the sequence. Bulk diffusion is minimized, and local concentrations are enhanced by channeling intermediate metabolites from one enzyme surface to the next (for reviews, see [9], [10], [13], [56], [57], [67], [68]). Channeling of metabolites can also be promoted among enzymes when they are bound near each other on structural elements, as observed in vitro with catalysts bound to carriers (e.g., see [66]) and as has been proposed for the tight coupling in vivo between ATP produced by glycolysis and ion-specific gates in cardiac muscle cells [65]. The rationale of catalytic efficiency provides an appropriate explanation for complexes that have been observed among enzymes that carry out consecutive reactions.

When enzymes catalyze key reactions (typically) at the beginnings and ends of unbranched pathways, complexes among such nonconsecutive enzymes can enhance the regulation of the entire pathway or system. Dysfunctional responses in branched pathways are avoided when complexes among such regulatory enzymes provide a balanced response among the several enzymes affected by a common regulatory molecule [37, 40]. Complexes among nonconsecutive reactions also can transmit important regulatory information via "short circuits" that effectively bypass the cause–effect sequence dictated by the intervening reactions [45, 53]. The rationale of regulatory effectiveness provides an appropriate explanation for complexes that have been observed among key enzymes that carry out nonconsecutive reactions.

The demonstration of functional advantages by rigorous analysis has gone hand in hand with experimental documentation of the widespread occurrence of enzyme–enzyme organization in cells. Far from being exceptional, one should expect a high degree of such organization to characterize the cytoplasm of all cells, and any formalism proposed for representing
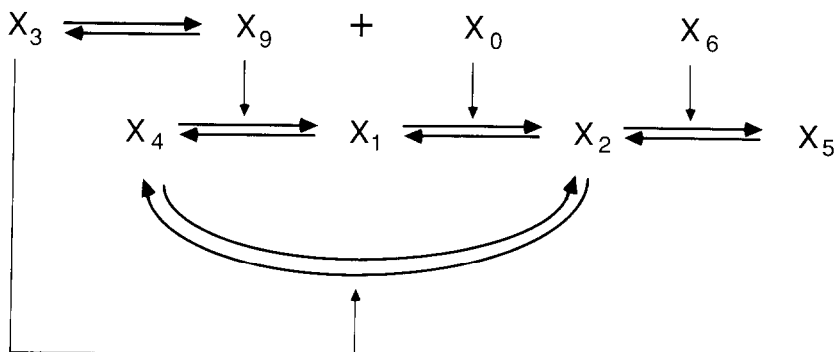
FIG. 1. Reference system that involves enzyme–enzyme interactions and channeling of metabolic flux. X_4 and X_5 are independent metabolite concentrations; X_1 and X_2 are dependent metabolite concentrations; X_6, X_9, and X_0 are concentrations of "free" enzyme; X_3 is the concentration of the multienzyme complex; $X_7 = X_3 + X_9$ is the total concentration of the first enzyme; and $X_8 = X_3 + X_0$ is the total concentration of the second enzyme. The numbering of concentration variables in this figure has been chosen to make the numbering in the final equations sequential: 1–3 for the dependent variables, and 4–8 for the independent variables. See text for discussion and Tables 1 and 2 for numerical values of parameters and nominal values of the variables in steady state.

realistic biochemical systems must be capable of dealing with this class of phenomena.

The Michaelis–Menten formalism (see footnote 1) that has dominated biochemical kinetics since the turn of the century did not anticipate this type of enzyme–enzyme organization. One of its fundamental assumptions has been that complexes do not occur between different forms of an enzyme or between different enzymes [4, 51, 52, 64, 70]. Although specific cases of enzyme–enzyme interaction have been treated in recent years by various modifications of the Michaelis–Menten formalism, no systematic formalism for dealing with this class of mechanisms has resulted from this approach. Mechanisms involving such complexes therefore provide an appropriate context for critically assessing any new formalism for the realistic characterization of complex biochemical systems.

As a paradigm for such mechanisms we shall consider the system in Figure 1. The enzymes X_9, X_0, and X_6 catalyze consecutive reactions that convert the initial substrate X_4 to the final product X_5. The enzymes X_9 and X_0 also can associate to form a multienzyme complex X_3, which is capable of catalyzing the conversion of the substrate X_4 to the intermediate X_2 without release of the bound intermediate X_1 [57]. The equilibrium between the free enzymes X_9 and X_0 and the complex X_3 is not influenced by the

binding of reactants. Thus,

$$K_{eq} = k_3/k_{-3} = X_3/X_9 X_0$$

at equilibrium, where X_i is the concentration of enzyme X_i.

The total concentration of the first enzyme will be distributed between its free form, X_9, and its complex form, X_3. Similarly, the total concentration of the second enzyme will be distributed between its free form, X_0, and its complex form, X_3. Since the total amount of each enzyme is conserved, the following relationships must hold:

$$X_7 = X_3 + X_9 \tag{1}$$

$$X_8 = X_3 + X_0. \tag{2}$$

Thus, the system in Figure 1 consists of 10 concentration variables; five of these (X_4, X_5, X_6, X_7, X_8) can be considered independent variables subject to direct experimental manipulation, and five (X_1, X_2, X_3, X_9, X_0) are dependent variables that can be manipulated only indirectly through change in the independent variables or parameters of the system.[3] In the power-law formalism there is one differential equation for each dependent variable. However, because of the constraints on total enzyme concentration [Eqs. (1) and (2)], only one of the three differential equations involving X_3, X_9, and X_0 is independent. Hence, the system's behavior is determined by the following three equations, which represent the conservation of mass:

$$\frac{dX_1}{dt} = v_{41} - v_{12} \tag{3}$$

$$\frac{dX_2}{dt} = v_{12} + v_{42} - v_{25} \tag{4}$$

$$\frac{dX_3}{dt} = v_{03} - v_{30} \tag{5}$$

where v_{ij} is the net forward rate of a process utilizing X_i for the production of X_j. The behavior of the other two dependent variables X_9 and X_0 can be obtained in turn from the constraints in Eqs. (1) and (2). The rate laws for each of the processes considered in Eqs. (3)–(5) can be derived by assuming a specific mechanism for each reaction. As a first approximation we shall

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[3] The distinction between direct manipulation of an independent variable and indirect manipulation of dependent variables is critical. Failure to observe these fundamental distinctions can lead to contradictions, as pointed out elsewhere [44].

consider each process to be described by a Michaelis–Menten equation for a reversible mechanism involving monomolecular reactants. The equations representing the system then become

$$\frac{dX_1}{dt} = \frac{X_9\left[(k_1/K_1)X_4 - (k_{-1}/K_{-1})X_1\right]}{1 + X_4/K_1 + X_1/K_{-1}}$$

$$- \frac{X_0\left[(k_2/K_2)X_1 - (k_{-2}/K_{-2})X_2\right]}{1 + X_1/K_2 + X_2/K_{-2}} \qquad (6)$$

$$\frac{dX_2}{dt} = \frac{X_0\left[(k_2/K_2)X_1 - (k_{-2}/K_{-2})X_2\right]}{1 + X_1/K_2 + X_2/K_{-2}}$$

$$+ \frac{X_3\left[(k_4/K_4)X_4 - (k_{-4}/K_{-4})X_2\right]}{1 + X_4/K_4 + X_2/K_{-4}}$$

$$- \frac{X_6\left[(k_5/K_5)X_2 - (k_{-5}/K_{-5})X_5\right]}{1 + X_2/K_5 + X_5/K_{-5}} \qquad (7)$$

$$\frac{dX_3}{dt} = k_3 X_9 X_0 - k_{-3} X_3 \qquad (8)$$

where

$$X_9 = X_7 - X_3 \qquad (9)$$

$$X_0 = X_8 - X_3. \qquad (10)$$

The nominal values for the independent variables and parameters are given in Table 1. These were selected to give a reasonable distribution of flux between the upper and lower branches and thereby avoid reduction of the system to a simple unbranched pathway. The corresponding nominal values for the dependent concentration variables and fluxes in steady state are given in Table 2. As can be seen, the net flow of material in the reference system under these conditions is always from the left to the right. Cases for which the direction of net flow changes are treated in detail elsewhere (see the paper immediately following this two-part series [55]).

This reference system will henceforth constitute our empirical reality. One could use the mathematical description for an individual process to mimic results produced by that process in a real system during a kinetic experiment in vitro. From such simulated kinetic data one could estimate the parameter values by using conventional methods (e.g., see [4]). Similarly, this reference system could be used to mimic results produced by a real system during

TABLE 1

Nominal Values for Independent Variables and Parameters
of the Reference System in Figure 1[a]

$K_1 = 20.0$	$k_1 = 2.77$	$X_4 = 10.0$
$K_{-1} = 13.3$	$k_{-1} = 0.922$	$X_5 = 2.00$
$K_2 = 5.00$	$k_2 = 8.66$	$X_6 = 10.0$
$K_{-2} = 30.0$	$k_{-2} = 17.3$	$X_7 = 20.0$
$K_4 = 1.00$	$k_4 = 2.74$	$X_8 = 15.0$
$K_{-4} = 6.67$	$k_{-4} = 3.04$	$k_3 = 0.300$
$K_5 = 10.0$	$k_5 = 5.50$	$k_{-3} = 1.00$
$K_{-5} = 17.8$	$k_{-5} = 1.96$	

[a] The units are micromolar (μM) for the Michaelis–Menten constants K_i and the concentrations X_i, s^{-1} μM^{-1} for the bimolecular elementary rate constant k_3, and s^{-1} for the monomolecular elementary rate constants k_i.

TABLE 2

Nominal Values for the Dependent Variables and Fluxes
in Steady State for the Reference System in Figure 1[a]

$X_1 = 5.00$	$v_{41} = 5.00$
$X_2 = 10.0$	$v_{12} = 5.00$
$X_3 = 11.0$	$v_{42} = 20.0$
	$v_{25} = 25.0$

[a] The units are micromolar (μM) for the concentrations X_i and μM s^{-1} for the fluxes v_{ij}.

experiments in vivo. For example, one could perform an experiment in which radioactive tracers are added to the system in steady state [69]. By measuring the specific radioactivity in each pool as a function of time and analyzing the data according to the well-established methods of compartmental analysis [20], one could estimate the steady-state values for the concentration variables and the fluxes in the system. One could change an independent variable, establish another steady state, and repeat the tracer experiment to determine new values for the concentrations and fluxes. In this way one could generate all of the systemic steady-state responses to changes in each of the independent concentration variables. The results of such in vivo experiments would appear as shown in Figure 2. In fact, these are simulated in vivo data produced by solving Eqs. (6)–(10).

This system will be our reference to which other approximate representations in the alternative theories will be compared. It has a distinct advantage over an actual biochemical system as reference in that we know precisely
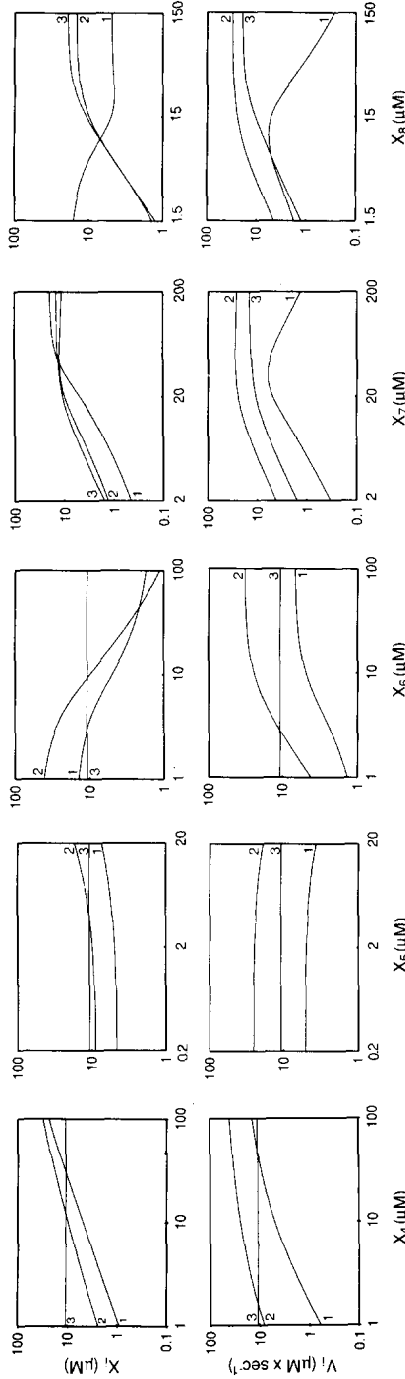
FIG. 2. The complete steady-state behavior of the reference system in Figure 1. The steady-state values for each dependent concentration variable (X_1, X_2, X_3) and flux (V_1, V_2, V_3) change in response to changes in each of the independent concentration variables (X_4, X_5, X_6, X_7, X_8). A single independent concentration variable is changed in each case, while the other independent variables have their nominal steady-state values. The response curves are identified by the subscripts of the corresponding dependent variables. Logarithmic scales are used for both axes. See text for discussion.

what the reality is. When discrepancies between different representations arise, these can be clearly understood, and there will be no possibility of attributing discrepancies to some putative complication that often can be postulated for a real biochemical system.

Finally, it should be noted that the reference system could be altered and made more realistic in a variety of ways (e.g., the association/dissociation of enzymes could be made to depend upon the concentration of various ligands, the rate laws could be made more complex functions of the reactant and/or enzyme concentrations, etc.), but the case we are considering is sufficient to illustrate the principal characteristics of this class of systems. It has the advantage of simplicity and yet it provides for critical experiments that clearly distinguish among BST, FOT, and MCT.

3. ANALYSIS USING BST: THE S-SYSTEM REPRESENTATION WITHIN THE POWER-LAW FORMALISM

The first step in analyzing a biochemical system such as that in Figure 1 according to BST is to represent the system in the S-system variant of the power-law formalism. There are straightforward rules for constructing this representation by inspection of the system [37, 40 (Chap 9), 61].

3.1. *MATHEMATICAL REPRESENTATION*

We start from the conservation of mass [Eqs. (3)–(5)]. Next the rate laws for the individual processes affecting each dependent variable X_i are grouped into two aggregate rate laws—one for net synthesis V_i and one for net degradation V_{-i}.

$$\frac{dX_1}{dt} = v_{41} - v_{12} = V_1 - V_{-1}$$

$$\frac{dX_2}{dt} = (v_{12} + v_{42}) - v_{25} = V_2 - V_{-2}$$

$$\frac{dX_3}{dt} = v_{03} - v_{30} = V_3 - V_{-3}.$$

At this point one writes the power-law representation for each of the aggregate rate laws; there will be one power-law function for each variable that directly influences the aggregate rate law in question. In the case of

Figure 1 we find

$$\frac{dX_1}{dt} = \alpha_1 X_1^{g_{11}} X_4^{g_{14}} X_9^{g_{19}} - \beta_1 X_1^{h_{11}} X_2^{h_{12}} X_0^{h_{10}}, \tag{11}$$

$$\frac{dX_2}{dt} = \alpha_2 X_1^{g_{21}} X_2^{g_{22}} X_3^{g_{23}} X_4^{g_{24}} X_0^{g_{20}} - \beta_2 X_2^{h_{22}} X_5^{h_{25}} X_6^{h_{26}}, \tag{12}$$

$$\frac{dX_3}{dt} = \alpha_3 X_9^{g_{39}} X_0^{g_{30}} - \beta_3 X_3^{h_{33}}, \tag{13}$$

where

$$g_{ij} = \left(\frac{\partial V_i}{\partial X_j}\right)_0 \left(\frac{X_j}{V_i}\right)_0 \tag{14}$$

$$h_{ij} = \left(\frac{\partial V_{-i}}{\partial X_j}\right)_0 \left(\frac{X_j}{V_{-i}}\right)_0 \tag{15}$$

$$\alpha_i = V_{i0} \prod_j X_{j0}^{-g_{ij}} \tag{16}$$

$$\beta_i = V_{-i0} \prod_j X_{j0}^{-h_{ij}}. \tag{17}$$

The parameters g_{ij} and h_{ij} are kinetic orders, and the parameters α_i and β_i are rate constants, familiar from chemical and biochemical kinetics. Note that because v_{12} appears both as V_{-1} and as the fraction v_{12}/v_{25} of V_2, two of the kinetic orders in Eqs. (11)–(13) are dependent upon the others. By the definition of kinetic order, these dependencies are seen to be [33, 49]:

$$g_{20} = h_{10}(v_{12}/v_{25})_0 \quad \text{and} \quad g_{21} = h_{11}(v_{12}/v_{25})_0.$$

The representation in Eqs. (11)–(13) is constructed about an operating point (signified by the additional subscript 0) and is an exact representation of the system at this point [33, 49]. Moreover, it provides a good approximation to the behavior of the system in a local neighborhood of this operating point [33, 62].

Because of the enzyme–enzyme interactions within the system, the enzyme concentrations X_3, X_9, and X_0 are not independent of each other. Thus, the power-law functions for these variables cannot be simply absorbed into the rate constant parameters α_i and β_i as they can in simple cases (see [49], [50]). In the reference system, the total concentrations of each enzyme (X_7 and X_8) are the independent variables, and these determine the concentrations of free (X_9 and X_0) and bound (X_3) enzymes through the associa-

tion/dissociation of X_9 and X_0. Thus, we can represent the constraints [Eqs. (9) and (10)] in the power-law formalism and then use the resulting equations to eliminate X_9 and X_0 from Eqs. (11)–(13). This is standard procedure in BST [33, 40 (Chap 15), 41, 49] to deal with aggregate variables or constraint relationships. In this case, the constraints in Eqs. (9) and (10) can be written

$$X_9 = \gamma_9 X_3^{f_{93}} X_7^{f_{97}} \tag{18}$$

and

$$X_0 = \gamma_0 X_3^{f_{03}} X_8^{f_{08}}, \tag{19}$$

where the parameters γ and f are analogous to the parameters α and g and are calculated in the same fashion, as indicated in Eqs. (14) and (16).

Substituting Eqs. (18) and (19) into Eqs. (11)–(13) yields the final S-system representation

$$\frac{dX_1}{dt} = \alpha_1' X_1^{g_{11}} X_3^{g_{13}} X_4^{g_{14}} X_7^{g_{17}} - \beta_1' X_1^{h_{11}} X_2^{h_{12}} X_3^{h_{13}} X_8^{h_{18}} \tag{20}$$

$$\frac{dX_2}{dt} = \alpha_2' X_1^{g_{21}} X_2^{g_{22}} X_3^{g_{23}'} X_4^{g_{24}} X_8^{g_{28}} - \beta_2 X_2^{h_{22}} X_5^{h_{25}} X_6^{h_{26}} \tag{21}$$

$$\frac{dX_3}{dt} = \alpha_3' X_3^{g_{33}} X_7^{g_{37}} X_8^{g_{38}} - \beta_3 X_3^{h_{33}} \tag{22}$$

where

$$\alpha_1' = \alpha_1 \gamma_9^{g_{19}}, \qquad \alpha_2' = \alpha_2 \gamma_0^{g_{20}}, \qquad \alpha_3' = \alpha_3 \gamma_9^{g_{39}} \gamma_0^{g_{30}},$$

$$\beta_1' = \beta_1 \gamma_0^{h_{10}}, \qquad g_{13} = g_{19} f_{93}, \qquad g_{17} = g_{19} f_{97}, \qquad h_{13} = h_{10} f_{03},$$

$$h_{18} = h_{10} f_{08}, \qquad g_{23}' = g_{23} + g_{20} f_{03}, \qquad g_{28} = g_{20} f_{08},$$

$$g_{33} = g_{39} f_{93} + g_{30} f_{03}, \qquad g_{37} = g_{39} f_{97}, \qquad g_{38} = g_{30} f_{08}.$$

With a little practice, these equations can be written directly from the mechanism. One notes which variables influence which aggregate processes and then follows the established convention for naming and numbering the parameters.

The parameter values can be determined either from a knowledge of the rate laws and the operating values when these are known [33, 36, 49], as is the case here, or by measurements of steady-state concentrations and fluxes in the intact system [36, 37, 40, 49; Sorribas and Savageau, in preparation], as can be simulated here. The resulting numerical characterization of the

reference system in BST is the following:

$$\frac{dX_1}{dt} = 2.44 \times 10^{-2} X_1^{-0.533} X_3^{-1.21} X_4^{1.07} X_7^{2.21} - 0.315 X_1^{2.57} X_2^{-2.14} X_3^{-2.71} X_8^{3.71}$$

$$\frac{dX_2}{dt} = 1.83 X_1^{0.514} X_2^{-0.685} X_3^{0.258} X_4^{0.320} X_8^{0.742} - 0.722 X_2^{0.568} X_5^{-0.0947} X_6^{1.00}$$

$$\frac{dX_3}{dt} = 7.52 \times 10^{-3} X_3^{-3.92} X_7^{2.21} X_8^{3.71} - X_3^{1.00}.$$

Specification of the independent variables X_4 through X_8, together with the initial values of the dependent variables X_1 through X_3, allows one to solve these equations for the subsequent behavior of the dependent variables.

3.2. BEHAVIOR OF THE CONCENTRATIONS IN STEADY STATE

The behavior of a complex biochemical system is characterized by the responses of the dependent variables to changes in the independent variables and parameter values of the system [37]. The S-system representation used in BST allows a complete characterization of the local behavior about the operating point for a biochemical system. With this characterization, one can predict the behavior of the dependent variables for a local change in any of the independent variables or any of the parameters of the model.

In particular, the steady-state behavior can be obtained directly from the steady-state solution. The explicit steady-state solution in symbolic form is readily obtained in BST [33, 37, 40]. First, set the time derivatives in Eqs. (20)–(22) to zero. Then rearrange the resulting algebraic equations and take logarithms to obtain the following linear equations in the dependent variables y_1, y_2, and y_3.

$$a_{11} y_1 - h_{12} y_2 + a_{13} y_3 = B_1$$
$$g_{21} y_1 + a_{22} y_2 + g'_{23} y_3 = B_2$$
$$a_{33} y_3 = B_3$$

where

$$b_i = \log(\beta_i/\alpha_i) \qquad B_1 = b_1 - g_{14} y_4 - g_{17} y_7 + h_{18} y_8$$
$$a_{ij} = g_{ij} - h_{ij} \qquad B_2 = b_2 - g_{24} y_4 + h_{25} y_5 + h_{26} y_6 - g_{28} y_8$$
$$y_i = \log X_i \qquad B_3 = b_3 - g_{37} y_7 - g_{38} y_8.$$

If the $n \times n$ system determinant $|A|$ is nonzero, i.e., if

$$|A| = a_{33}(a_{11} a_{22} + g_{21} h_{12}) \neq 0, \tag{23}$$

then one can solve explicitly for y_1, y_2, and y_3. That is, Eq. (23) is a general

condition for the very existence of a steady state, and it provides an important constraint on the permissible values for the parameters of the model [33, 49].

The solution can be written in standard matrix notation [33, 36], which for each of the variables is equivalent to

$$y_i = \sum_{j=1}^{3} M_{ij} b_j + \sum_{k=4}^{8} L_{ik} y_k, \qquad i = 1, 2, 3, \tag{24}$$

where

$M_{11} = a_{22} a_{33} / |A|, \qquad M_{12} = h_{12} a_{33} / |A|, \qquad M_{13} = -(h_{12} g'_{23} + a_{13} a_{22}) / |A|,$

$M_{21} = -g_{21} a_{33} / |A|, \qquad M_{22} = a_{11} a_{33} / |A|, \qquad M_{23} = (g_{21} a_{13} - g'_{23} a_{11}) / |A|,$

$M_{31} = M_{32} = 0, \qquad M_{33} = (a_{11} a_{22} + g_{21} h_{12}) / |A|,$

$L_{14} = -(a_{22} a_{33} g_{14} + h_{12} a_{33} g_{24}) / |A|, \qquad L_{15} = h_{12} a_{33} h_{25} / |A|,$

$L_{16} = h_{12} a_{33} h_{26} / |A|,$

$L_{17} = \left[-a_{22} a_{33} g_{17} + (g'_{23} h_{12} + a_{13} a_{22}) g_{37} \right] / |A|$

$L_{18} = \left[a_{22} a_{33} h_{18} - h_{12} a_{33} g_{28} + (g'_{23} h_{12} + a_{13} a_{22}) g_{38} \right] / |A|$

$L_{24} = (g_{21} a_{33} g_{14} - a_{11} a_{33} g_{24}) / |A|, \qquad L_{25} = a_{11} a_{33} h_{25} / |A|,$

$L_{26} = a_{11} a_{33} h_{26} / |A|,$

$L_{27} = \left[g_{21} a_{33} g_{17} - (g_{21} a_{13} - g'_{23} a_{11}) g_{37} \right] / |A|$

$L_{28} = \left[-g_{21} a_{33} h_{18} - a_{11} a_{33} g_{28} - (g_{21} a_{13} - g'_{23} a_{11}) g_{38} \right] / |A|$

$L_{34} = L_{35} = L_{36} = 0, \qquad L_{37} = -(a_{11} a_{22} + g_{21} h_{12}) g_{37} / |A|,$

$L_{38} = -(a_{11} a_{22} + g_{21} h_{12}) g_{38} / |A|.$

The explicit steady-state solution, expressed by Eq. (24), completely characterizes the steady-state behavior of the system in the neighborhood of the steady state, and it allows one to study the response of the system to change in each of its constitutive elements. One can determine three distinct types of responses: (1) the changes in the dependent variables (X_1, X_2, and X_3) evoked by changes in the independent variables, (2) the corresponding changes evoked by changes in the rate constants, and (3) the changes evoked by changes in the kinetic orders of the model.

Logarithmic Gains. The change in any dependent variable (say X_1) that results from a 1% change in any independent variable (say X_6) is given by
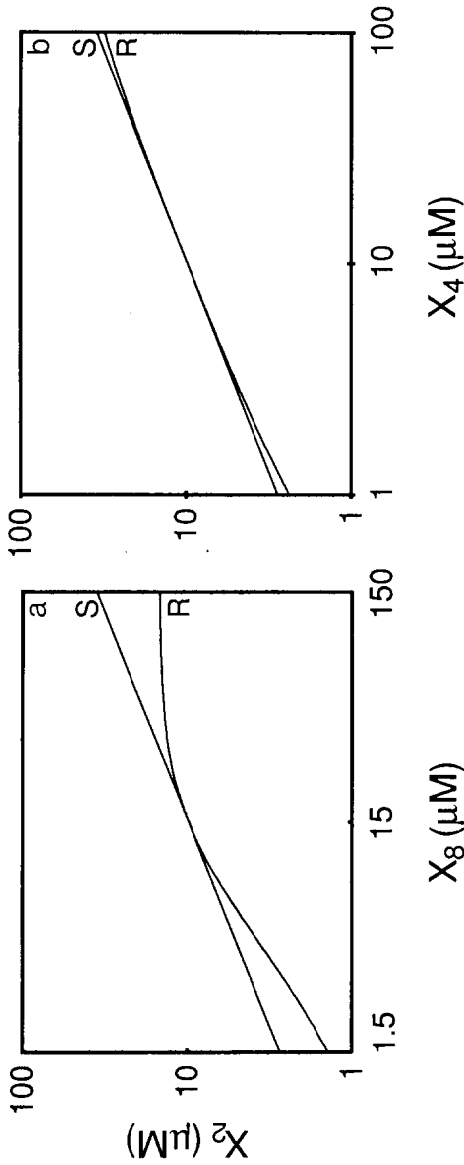
FIG. 3. Comparison of steady-state behavior predicted by the S-system representation within BST and the actual behavior of the reference system in Figure 1, showing representative responses with (*a*) a narrow range of agreement and (*b*) a wide range of agreement. The behavior predicted using the S-system variant within BST and the empirically determined behavior of the reference system are indicated by S and R, respectively. Logarithmic scales are used for both axes. For further comparisons, see summary of results in Table 6.

the appropriate logarithmic gain [36, 40, 50][4]

$$L(X_1, X_6) = \frac{\partial y_1}{\partial y_6} = L_{16} = \frac{h_{12}a_{33}h_{26}}{|A|} \qquad (25)$$

This is calculated from Eq. (24) by taking the appropriate derivative, and the result, which can be seen by inspection, is the numerical coefficient preceding y_6 in Eq. (24). The logarithmic gain can be interpreted geometrically as the slope of y_i versus y_k at the nominal steady-state operating point of the system [36]. (For example, see Figure 3.)

Rate-Constant Sensitivities. The change in such a variable that results from a 1% change in any rate-constant parameter (say β_3) is given by the appropriate parameter sensitivity [35, 40, 50]

$$S(X_1, \beta_3) = \frac{\partial X_1}{\partial \beta_3} \frac{\beta_3}{X_1} = M_{13} = -\frac{g'_{23}h_{13} + a_{13}a_{22}}{|A|}. \qquad (26)$$

This also is calculated from Eq. (24) by taking the appropriate derivative, and the result, which can be seen by inspection, is the numerical coefficient preceding b_3 in Eq. (24). According to the meaning of the parameter b_j, it follows immediately that

$$S(X_i, \alpha_j) = -M_{ij} = -S(X_i, \beta_j).$$

Kinetic-Order Sensitivities. The change in a dependent variable that results from a 1% change in any kinetic-order parameter (say g'_{23}) is given by the analogous parameter sensitivity

$$S(X_1, g'_{23}) = \frac{\partial X_1}{\partial g'_{23}} \left(\frac{g'_{23}}{X_1} \right)$$

$$= S(M_{13}, g'_{23}) M_{13}b_3 + S(L_{17}, g'_{23}) L_{17}y_7 + S(L_{18}, g'_{23}) L_{18}y_8,$$

$$(27)$$

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[4]For simplicity, we shall drop the zero subscript, since it is understood that the variables, functions, and derivatives are evaluated at the operating point.

or, as indicated elsewhere [36, 40, 50], one can express the sensitivities with respect to kinetic orders in terms of the logarithms of the dependent variables

$$S(y_1, g'_{23}) = S(X_1, g'_{23})/y_1,$$

and then the sensitivity has a convenient interpretation as the weighted average of the sensitivities of the M and L coefficients of the system. These sensitivities are readily calculated from the solution in Eq. (24). According to the meaning of the parameters $a_{jp}(=g_{jp}-h_{jp})$, it also follows that

$$S(X_i, g_{jp})/g_{jp} = -S(X_i, h_{jp})/h_{jp}, \qquad i, j = 1,2,3; \ p = 1,2,\ldots,8.$$

3.3. BEHAVIOR OF THE FLUXES IN STEADY STATE

The fluxes through the pools of the system (V_i and V_{-i}) follow directly from the solution of the dependent concentration variables [33, 49]. From Eqs. (20)–(22) and (24) in steady state, one can write

$$\log V_i = \log V_{-i} = \log \alpha'_i + \sum_{k=1}^{3} \left(\sum_{j=1}^{3} g_{ij} M_{jk} \right) b_k$$

$$+ \sum_{k=4}^{8} \left(g_{ik} + \sum_{j=1}^{3} g_{ij} L_{jk} \right) y_k, \qquad i = 1,2,3, \tag{28}$$

where $g_{12} = g_{15} = g_{16} = g_{18} = g_{25} = g_{26} = g_{27} = g_{31} = g_{32} = g_{34} = g_{35} = g_{36} = 0$, and the M_{jk} and L_{jk} factors are given in Eq. (24).

Logarithmic Gains. The change in any flux (say V_1) that results from a 1% change in any independent variable (say X_6) is given by the appropriate logarithmic gain,

$$L(V_1, X_6) = \frac{\partial(\log V_1)}{\partial(\log X_6)} = g_{11} L_{16} = \frac{g_{11} h_{12} a_{33} h_{26}}{|A|}. \tag{29}$$

By inspection, this is simply the coefficient preceding y_6 in the first of Eqs. (28). Alternatively, since X_6 is not present in V_1 [see Eq. (20)], its influence on V_1 must be exerted via the dependent variables X_1 and X_3. This influence is the kinetic order with respect to X_1 times the logarithmic gain L_{16} plus the kinetic order with respect to X_3 times the logarithmic gain L_{36}, or $g_{11} L_{16}$ since $L_{36} = 0$ in this case.

Rate-Constant Sensitivities. The change in such a flux that results from a 1% change in any rate-constant parameter (say β_3) is given by the appropri-

ate parameter sensitivity

$$S(V_1, \beta_3) = \frac{\partial V_1}{\partial \beta_3}\left(\frac{\beta_3}{V_1}\right) = -S(V_1, \alpha_3)$$

$$= g_{11}M_{13} + g_{13}M_{33} = g_{11}S(X_1, \beta_3) + g_{13}S(X_3, \beta_3). \quad (30)$$

This follows by inspection of Eq. (28), or by noting that β_3 has no direct influence on V_1 [see Eq. (20)] and therefore its influence is the kinetic order with respect to X_1 (g_{11}) times $S(X_1, \beta_3) = M_{13}$ plus the kinetic order with respect to X_3 (g_{13}) times $S(X_3, \beta_3) = M_{33}$.

Kinetic-Order Sensitivities. The change in a flux that results from a 1% change in a kinetic-order parameter (say g'_{23}) is given by the analogous parameter sensitivity

$$S(V_1, g'_{23}) = \frac{\partial V_1}{\partial g'_{23}}\left(\frac{g'_{23}}{V_1}\right) = g_{11}S(X_1, g'_{23}). \quad (31)$$

Again, this could be expressed as the weighted average of the indirect influences of g'_{23}, in this case exerted only via changes in X_1.

The flux relationships in each instance are seen to be simply sums of the corresponding concentration relationships multiplied by appropriate kinetic orders. Hence, the flux relationships may be considered secondary and can be produced easily once the concentration relationships have been determined.

Other Relationships in Steady State. The well-known orthogonality properties that are inherent in the explicit symbolic solution of any biochemical system in BST also imply a number of other relationships among the kinetic orders of the system and the systemic coefficients—logarithmic gains and parameter sensitivities. These relationships, which are described in detail elsewhere (see [47]), also are secondary in the sense that one can obtain the explicit solution that completely characterizes a biochemical system and never explicitly invoke these relationships [33, 50]. We shall return to some of these relationships in the second paper of this series [54].

3.4.   *BEHAVIOR ABOUT THE NOMINAL STEADY STATE*

BST provides an explicit symbolic condition that is necessary for the local stability of the steady state [39, 40]; namely,

$$(-1)^n |A| > 0,$$

which in the case of the reference system becomes

$$a_{33}(a_{11}a_{22} + g_{21}h_{12}) < 0.$$

TABLE 3

Logarithmic Gains: Percentage Change in the Dependent Variables
of the System in Response to a 1% Change in an Independent Variable[a]

Independent variable	Dependent variable					
	X_1	X_2	X_3	V_1	V_2	V_3
X_4	0.726	0.553	0.00	0.683	0.314	0.00
X_5	0.0727	0.105	0.00	-0.0387	-0.0348	0.00
X_6	-0.768	-1.11	0.00	0.410	0.368	0.00
X_7	1.39	0.661	0.449	0.928	0.375	0.449
X_8	-0.440	0.567	0.754	-0.678	0.322	0.754

[a] Determined for either the reference system or its S-system representation within BST.

This is essential for interpreting steady-state predictions for complex systems (e.g., see [37], [40], [50]); it also is an important constraint on the permissible values for the parameters of the model. One can verify easily that the parameter values in the S-system representation are consistent with the stable steady state exhibited by the reference system.

The systematic structure of the S-system representation within BST has led to the development of several new methodologies for evaluation and analysis of complex biochemical systems [17, 19, 37, 40, 61]. The most recent developments dramatically increase one's ability to explore the dynamic behavior of intact systems. The local dynamic behavior of biochemical systems is determined by solving the differential equations that represent the system in BST [Eqs. (20)–(22)]. This is routinely accomplished with a menu-driven user-friendly program that has been under continuous development since the late 1960s [17, 19, 34, 40, 61; Irvine and Savageau, in preparation]. The current version, called ESSYNS (for Evaluation and Simulation of Synergistic Systems), runs on an IBM PC/AT. The program includes state-of-the-art methods for solving differential equations [19; Irvine and Savageau, in preparation], the complete steady-state analysis described above, graphical presentation and analysis, as well as full data management facilities [Voit, Irvine and Savageau, in preparation].

4. RESULTS

4.1. LOGARITHMIC GAINS

The changes of the dependent variables X_1, X_2, and X_3 (and consequently V_1, V_2, and V_3 as well) with respect to changes in the independent variables of the system (X_4, X_5, X_6, X_7, X_8) at the steady state are given by the appropriate logarithmic gains and are summarized in Table 3. One sees a

trend with the independent variables X_7 and X_8 having the greatest effect and X_5 the least effect on any given dependent variable. The values in this table are equal to the logarithmic gains as calculated from Eqs. (25) and (29) or, in the case of the concentrations, as obtained by inspection of the explicit solution [Eq. (24)], and represent the slopes of the steady-state responses at the operating point. Clearly these steady-state responses could also be determined operationally from the corresponding measurements on the intact reference system. Such measurements provide the information neces-sary for estimating kinetic orders and rate constants and thus for construct-ing the S-system representation. When the logarithmic gains predicted by BST are compared with those measured at the operating point for the reference system itself, one obtains exactly the same values (e.g., see Figure 3). The straight lines in Figure 3 are not simply extrapolations of the logarithmic gains at the operating point but are in fact the explicit steady-state solutions in BST. These solutions and those for the reference system are in close agreement, provided the excursions of the independent variables from the nominal steady state operating point are not too large.

Eventually, the discrepancies for large variations become apparent. This is inherent in the nature of all representations. The issue in comparing alternative representations is which one remains valid over the widest range of variations. We shall return to this point in the following papers [54, 55].

4.2. PARAMETER SENSITIVITIES

Another important aspect of characterizing biochemical systems is the parameter sensitivity of the corresponding model. These quantities are useful for predicting changes in system behavior that result from actual changes in parameter values, either by mutation or by changes in physical conditions [27, 35–37, 40, 46, 50].

The complete set of parameter sensitivities for the reference system are given in Table 4 (rate-constant parameters) and Table 5 (kinetic-order parameters). These results can be calculated from Eqs. (26), (27), (30), and (31) or in some cases obtained directly from the explicit solution in Eq. (24). The results in Table 4 show the tendency for a change in the rate constants α_2' or β_2 to have the greatest effect on the dependent variables, with the exception of X_3 and V_3, which are uncoupled from these effects. The results in Table 5 indicate the relative importance of the kinetic orders with respect to the independent variables X_7 and X_8, while that with respect to X_5 is relatively unimportant.

Although one can determine mathematically the consequence of a change in any individual parameter, physical or genetic alteration of a system

TABLE 4

Rate-Constant Sensitivities: Percentage Change in the Dependent Variables
of the System in Response to a 1% Change in a Rate Constant

Rate constant	Dependent variable					
	X_1	X_2	X_3	V_1	V_2	V_3
α_1'	0.450	0.184	0.00	0.760	0.105	0.00
β_1'	-0.450	-0.184	0.00	0.240	-0.105	0.00
α_2'	0.768	1.11	0.00	-0.410	0.632	0.00
β_2	-0.768	-1.11	0.00	0.410	0.367	0.00
α_3'	0.177	0.115	0.203	-0.340	0.0651	0.203
β_3	-0.177	-0.115	-0.203	0.340	-0.0651	0.797

TABLE 5

Kinetic-Order Sensitivities: Percentage Change in the Dependent Variables
of the System in Response to a 1% Change in a Kinetic Order

Kinetic order	Dependent variable					
	X_1	X_2	X_3	V_1	V_2	V_3
g_{11}	-0.386	-0.158	0.00	-0.653	-0.0899	0.00
g_{13}	-1.30	-0.534	0.00	-2.20	-0.303	0.00
g_{14}	1.11	0.454	0.00	1.87	0.258	0.00
g_{17}	2.98	1.22	0.00	5.03	0.693	0.00
h_{11}	-1.86	-0.763	0.00	0.991	-0.433	0.00
h_{12}	2.21	0.908	0.00	-1.18	0.516	0.00
h_{13}	2.92	1.20	0.00	-1.55	0.680	0.00
h_{18}	-4.52	-1.85	0.00	2.41	-1.05	0.00
g_{21}	0.635	0.922	0.00	-0.339	0.523	0.00
g_{22}	-1.21	-1.75	0.00	0.645	-0.997	0.00
g_{23}'	0.474	0.687	0.00	-0.253	0.390	0.00
g_{24}	0.566	0.820	0.00	-0.301	0.466	0.00
g_{28}	1.54	2.24	0.00	-0.822	1.27	0.00
h_{22}	-1.00	-1.46	0.00	0.535	0.481	0.00
h_{25}	0.0504	0.0731	0.00	-0.0269	-0.0241	0.00
h_{26}	-1.77	-2.57	0.00	0.942	0.845	0.00
g_{33}	-1.66	-1.08	-1.91	3.19	-0.611	-1.91
g_{37}	1.17	0.758	1.35	-2.25	0.431	1.35
g_{38}	1.78	1.15	2.04	-3.42	0.654	2.04
h_{33}	-0.424	-0.274	-0.487	0.815	-0.156	1.91

generally affects several component parameters simultaneously [27, 35–37, 40, 46, 50].[5] There are two steps required for accurate prediction of the systemic consequences of a small specific mutation or physical change affecting a given process. First one must determine the change in all relevant parameters of the affected process, and second one must add the changes multiplied by the sensitivities with respect to each of these parameters to predict the net change in a systemic property. For example, suppose one knows that a mutation has occurred in the structural gene for the enzyme X_6. One could do experiments, either in vitro or in vivo, to determine how the component parameters $(\beta_2, h_{22}, h_{25}, h_{26})$ of the reaction have changed. By knowing these changes and the sensitivities in Tables 4 and 5, one can predict the net change in a given systemic property, say X_2.

A second use of parameter sensitivities, which is equally important but perhaps less emphasized, is in characterizing the quality of a model. Quality is measured by the extent to which a model accurately characterizes a system. For example, a model with low parameter sensitivities will hold for larger variations than one with high sensitivities (for further discussion, see Sorribas and Savageau [55]). This can be understood intuitively. When the concentration variables of a system vary from one steady-state value to another, the operating points change, and hence the values of component parameters in the model may change. If the sensitivities of these parameters are low, then changes will have only a minor influence on systemic behavior; a concentration variable can experience a large change and still the model will accurately predict systemic behavior. In other words, the range of accuracy for a model is larger when parameter sensitivities are lower. Since parameters with high sensitivities exert greater influence on system behavior, these sensitivities are important guides to phenomena or processes that merit more intense experimental scrutiny (e.g., see [27]).

The distinction between parameters and variables [37, 40] must be kept clearly in mind. This is particularly true because a given quantity considered under certain circumstances to be a parameter may under other circumstances be considered a significant variable in the system, and it becomes appropriate to change its definition to that of a variable. Any process that is influenced by such a variable then will have an additional term in its Taylor

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[5]For example, in a simple Michaelis–Menten rate law, change cannot occur in the K_m for substrate alone. Of necessity, change occurs in at least one other parameter—the K_m for product, the maximal velocity in the forward direction, or the maximal velocity in the reverse direction—and the changes cannot be unrelated to each other since the Haldane relationships must be satisfied. This is well known to enzymologists (e.g., see [4]) but has seldom been considered in attempts to account for the systemic consequences of a given alteration in a component of the system.

series expansion and an additional power law in the corresponding product
of power laws that constitute its power-law representation [40 (Chap 15)]. In
some cases, the parameter might become an independent variable. For
example, in a system with a simple rate law given by

$$v_i = V_{mi} X_1 / (X_1 + K_{mi}) \approx \alpha'_i X_1^{g_{i1}},$$

one can define the K_{mi} as an independent variable X_k. Then

$$v_i = V_{mi} X_1 / (X_1 + X_k) \approx \alpha_i X_1^{g_{i1}} X_k^{g_{ik}}$$

where, as usual, $g_{ik} = (\partial v_i / \partial X_k)(X_k / v_i)$. The systemic consequences of a
change in the independent variable X_k can be ascertained in the conven-
tional manner by calculating the appropriate logarithmic gains. In other
cases, the parameter might become a dependent variable. For example,
temperature is considered a physical parameter in many systems, but in
systems with a strongly exothermic reaction, temperature is more appropri-
ately considered a dependent variable [59]. Temperature T then is defined as
a variable X_j, which appears as a typical dependent variable in the power-law
formalism [48].

4.3. BEHAVIOR ABOUT THE NOMINAL STEADY STATE

Predicted steady-state responses in the local neighborhood of the nominal
operating point have been determined analytically as well as numerically by
using ESSYNS; the same results are obtained in each case. For relatively
wide variations about the steady state, these results agree with the actual
responses of the reference system, as determined by numerical solution of
Eqs. (6)–(10), again by using ESSYNS. Representative results exhibiting a
narrow or a wide range of agreement are shown graphically in Figure 3. The
range over which predicted and actual responses agree to within 10% is
summarized in Table 6 for all responses to all independent variables. As
expected from the results in Tables 3–5, the reference system tends to be
more accurately represented for changes in X_5 and less accurately repre-
sented for changes in X_7 and X_8. The range of validity is greater than
90-fold in the best cases, and in the worst cases is never smaller than 2- to
3-fold, with an average range of 20-fold. This range compares well with the
range of variation exhibited by concentrations in typical in vivo preparations
(see Section 5). By contrast, typical linear representations have ranges
measured in percentage rather than fold variation.

The results in the preceding paragraph demonstrate the accuracy of the
S-system representation in predicting a final steady state in response to a
change in independent variables. By using Eqs. (6)–(10), one also can obtain

TABLE 6

Range of Concentrations Over Which the S-System Representation
Within BST is Accurate[a]

Independent variable	Dependent variable					
	X_1	X_2	X_3	V_1	V_2	V_3
X_4	> 97.5	55.6	∞[b]	6.27	13.6	∞
X_5	48.5	27.1	∞	58.2	61.3	∞
X_6	7.44	10.1	∞	4.22	4.06	∞
X_7	4.37	2.76	3.34	1.98	3.21	3.34
X_8	2.58	3.38	4.11	1.96	4.10	4.11

[a] The range is measured by the ratio of the largest to the smallest values of the independent variable that leave the dependent variable within 10% of its actual value. The larger this range, the greater the accuracy of the representation.
[b] The dependent variables X_3 and V_3 are not influenced by changes in the independent variables X_4, X_5, X_6.

the transient response between two steady states. A typical dynamic response to a change within the local neighborhood of the steady state is shown in Figure 4. Initially the system is in a steady state. At $t = 5$ s the system is perturbed by increasing the independent concentration X_8 from 15 to 25 μM (Fig. 4a). This is accomplished by adding free enzyme X_0. According to the constraints expressed by Eqs. (1) and (2), X_8 and X_0 increase by the same amount. With time the concentrations X_9 and X_0 decrease toward equilibrium as the free enzyme molecules combine to form the complex, and the concentration X_3 increases by the corresponding amount (Fig. 4b, c, d). Accordingly, the intermediate concentrations X_1 and X_2 change to a new steady state determined by the new values of the independent variables. The concentration of the first intermediate X_1 decreases due to the drop in free enzyme X_9 and also due to the rise in free enzyme X_0 (Fig. 4e). The concentration of the second intermediate X_2 increases (Fig. 4f) because the increase in flux from X_4 (positive logarithmic gain for $V_2 - V_1$ in Table 3) is greater than the decrease in flux from X_1 (negative logarithmic gain for V_1 in Table 3).

After the system has reached a new steady state, at $t = 10$ s the total concentration of the second enzyme X_8 is abruptly changed from 25 back to 15 μM. There is a proportionate drop in the concentrations X_0 and X_3, and the drop in X_3 is matched by an increase in X_9. With time the concentrations X_9 and X_0 decrease toward equilibrium as the free enzyme molecules combine to form a complex, and concentration X_3 increases by the corresponding amount (Fig. 4b, c, d). The responses of X_1 and X_2 follow accordingly.
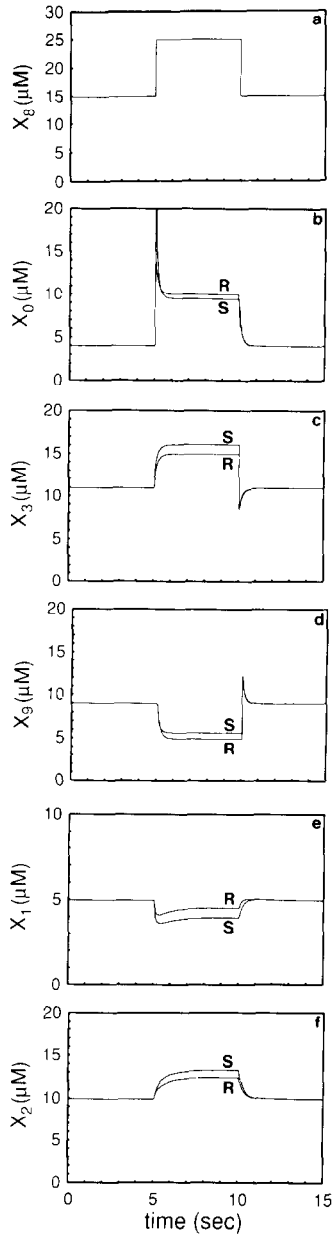
FIG. 4. Dynamic response to a change within the local neighborhood of the steady state. Before time equals 5 s the system is in steady state. At time equals 5 s the independent concentration X_8 is increased from 15 to 25 μM by the addition of free enzyme X_0. At time equals 10 s the concentration of X_8 is decreased from 25 to 15 μM. The responses predicted by the S-system variant within BST and the empirically deter-mined responses of the reference system are indicated by S and R, respectively.

Overshoots in the dynamic response of X_1 result from the initial perturbation in X_0 and the overshoot in X_9 when the perturbation is removed. When X_8 is increased there is a momentary excess in the concentration of free enzyme X_0 relative to X_9, which causes depletion of the intermediate X_1. When X_8 is decreased there is a momentary excess in the concentration of free enzyme X_9 relative to X_0, which causes accumulation of the intermediate X_1. These transient imbalances are eliminated as the enzyme–enzyme interactions relax to equilibrium.

These results are in reasonable agreement with the empirical data obtained directly from the reference system. The maximum error between responses is 5–10%. Similar results are obtained by perturbation of other independent variables (data not shown).

These results illustrate the value of an explicit representation for the kinetic equations describing a system. For instance, X_2 in the reference system was correctly predicted to increase as a result of the perturbation in X_8. Without an explicit representation, one cannot predict, even qualitatively, the dynamic responses of a complex system. In fact, X_2 can increase or decrease depending on the parameter values of the actual system. A qualitative analysis of the system in Figure 1 could not have predicted that X_2 would increase.

5. DISCUSSION

If one were to identify the most outstanding characteristic of BST, it would be its ability to yield explicit steady-state solutions in symbolic form [33]. Such solutions are rare for complex nonlinear systems, but when they exist, important consequences follow.

The existence of symbolic solutions for different systems being compared allows one to equate specific systemic responses while exploring the implications of alternative values for their component parameters. This provides the mathematical equivalent of a "well-controlled" experiment [17, 37, 40, 43]. Such analyses with symbolic solutions often lead to very general conclusions that are independent of the particular numerical values associated with the parameters of a specific system (e.g., see [17], [18], [38], [40], [45]). Such analyses have succeeded where others requiring numerical values have not because numerical values often are unknown and in some cases are difficult or impractical to obtain experimentally. Although symbolic analysis is generally more difficult, the rewards are correspondingly greater.

Symbolic analysis of control in biochemical pathways by the use of BST has previously led to the *prediction* of specific enzyme–enzyme interactions [37, 40, 45, 53] that have been confirmed experimentally, which demonstrates the power of this approach. However, a complete and rigorous *numerical* analysis of a specific model of enzyme–enzyme interactions has not been

published. This paper provides the complete analysis of such a system containing enzyme complexes. The results make it evident that BST provides a well-structured theory for analysis of complex biochemical systems, including those with enzyme–enzyme interactions.

Within the framework of the power-law formalism, the steady-state analysis using the S-system representation is complete. The influence of every independent concentration variable (Table 3) and every parameter value (Tables 4 and 5) on every dependent variable of the system has been accounted for. These influences are determined not only for the single steady state considered in Table 2, but also for all steady states in its local neighborhood (Fig. 3).

The dynamic responses of the system within the local neighborhood of the steady state also have been characterized. Although intuitively one could anticipate that addition of one of the two enzymes that form a complex reduces the free concentration of the other, the consequences of this in the intact system are much less certain. Even *qualitative* behavior—for example, whether X_2 will increase, decrease, or remain unchanged—depends upon the *numerical* values of component parameters (data not shown). Prediction of *quantitative* aspects of the response is nearly impossible without a systematic, quantitative approach such as that provided by BST. As seen in Figure 4, BST provides reasonably accurate predictions for transient responses of the system following changes in the independent variable X_8 (average error approximately 5%). Comparable results are obtained following changes in other independent variables of the system.

The analysis in this paper has provided further evidence for the accuracy of the power-law formalism. Direct comparison with the actual behavior of the reference system (Table 6 and Figs. 3 and 4) shows the range of variation in the independent concentration variables for which the S-system representation within BST is accurate. The range varies from a minimum of about 2-fold to a maximum greater than 90-fold, with an average range of 20-fold. Previous examination of isolated processes has shown a considerable range of variation in the independent concentrations for which the power-law formalism is accurate [31, 33, 62]. Evidence from intact systems, consisting of many processes, that have been driven experimentally beyond physiological ranges often shows an even wider range, as large as 100- to 1000-fold, with accurate representation by the power-law formalism [36, 37, 40, 41, 60]. Reasons for the increased accuracy within intact systems are discussed in [40], [42], and [62]. This degree of accuracy with power-law representation is considerably greater than that with linear representations, which is typically measured in percentage rather than fold variation.

The range of accurate representation provided by the power-law formalism is broad enough to encompass the typical physiological range of varia-

tion, and perhaps much of the relevant pathological range as well. Although systematically collected data relevant to this point are few, there are abundant data for many biochemical and cellular variables in humans that have been collected in hospitals throughout the world. These variables include biochemical concentrations and fluxes, cellular concentrations and turnover rates, and concentrations of therapeutic agents. The range of variation seen in a few major hospitals has been tabulated for each of these variables (e.g., see [28], [63]). For a wide variety of metabolites, the range can be small. For example, sodium and fasting glucose variations are no greater that 7% and 69%, respectively. Examples of metabolites that exhibit a larger range are aldosterone (16-fold) and 17-hydroxyprogesterone (30-fold). On average, the range is 3.9-fold for 160 variables. Of course, all these ranges may be considerably greater than one would find in the normal population, since they are biased toward the extremes in a clinical setting. More detailed data, including dynamic responses, from clinical studies gives the same general picture [3]. Many metabolites have ranges around 2-fold, with hormones tending to have the highest normal ranges (typically 5-fold, but occasionally as high as 10- to 100-fold) as well as the highest pathological ranges (up to 10,000-fold for some tumors). The average range over a wide variety of metabolites is about 3–5-fold. (We thank Dr. D. H. Irvine of The University of Michigan Medical School for making these data known to us.) The general conclusion that can be drawn by comparing the above experimental data with the results for BST in this paper and elsewhere is that the actual ranges and the ranges of accurate representation in BST are roughly the same. Although there undoubtedly will be cases in which the range of variation for a specific metabolite will be larger than can be accurately represented by the power-law formalism, this remains to be explored in specific cases. Hence, the notion that the power-law representation is an inappropriate approximation, too crude to be of use for real biochemical systems, is clearly without basis.

In conclusion, the concepts, theory, and methodology already developed in BST provide a very general framework for analyzing complex biochemical systems. Their utility has been demonstrated in a broad range of applications. In this paper we have shown that the S-system variant within BST also represents enzyme–enzyme interactions in a systematically structured fashion that greatly facilitates analysis of complex biochemical systems in which these interactions play a prominent role. This representation captures the essential character of the underlying nonlinear processes over a wide range of variation in the independent variables of the system. It is important to point out that the parameters of the S-system are obtained directly from a small number of experimental measurements on the intact system (logarithmic gains for concentrations and fluxes) and that this process does not

require detailed characterization of the underlying enzymatic mechanisms for each reaction. This methodology can greatly facilitate experimental characterization of complex systems.

In the following paper (Part II) we shall turn to the comparison of alternative theories based upon the power-law formalism. The system in Figure 1 provides a discriminating test that allows various theories within this formalism to be clearly distinguished and evaluated.

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