J. theor. Biol. (1989) **141**, 93–115

Constraints Among Molecular and Systemic Properties: Implications for Physiological Genetics

MICHAEL A. SAVAGEAU† AND ALBERT SORRIBAS‡

Department of Microbiology and Immunology, The University of Michigan, Ann Arbor, Michigan 48109-0620, *U.S.A.*

(*Received* 1 *April* 1989, *and accepted in revised form* 12 *June* 1989)

Physiological genetics attempts to relate the molecular genetic properties of an organism—the genotype—to its integrated or physiological behavior—the phenotype. There has been relatively little progress in this field when compared to the neighboring fields of molecular and population genetics. This is due in part to the large number of highly non-linear interactions that characterize such systems. Biochemical Systems Theory is one approach that shows promise in dealing with the large number of non-linear interactions in a systematically structured manner. A variant of this approach has stressed the use of specific mathematical constraints, called summation and connectivity relationships, among molecular and systemic properties. In particular, the summation relationship has been used to argue that the predominance of recessive mutations is the inevitable consequence of the kinetic structure of enzyme networks and need not be attributed to natural selection. In order to put in broader perspective the implications of such constraints for physiological genetics, we have presented in this paper the outlines of the larger theory and the set of generalized steady state constraints that follow from first principles within this theory. The results show that the summation relationship suffers from a number of fundamental limitations that make it invalid for analyzing realistic biological systems. It also is shown that the more general constraint relationships, while valid, provide nothing new that cannot be obtained directly from the explicit solutions that are available within the larger theory. Thus, one can conclude that approaches based directly on the underlying equations of the system are superior to those based upon constraint relationships as a foundation for the development of physiological genetics.

1. Introduction

The rapid advances in molecular genetics during the past several decades have yielded an abundance of information about the molecular determinants of biological systems. Molecular advances also have provided new techniques that have stimulated the field of population genetics. At the interface between these major disciplines stands physiological genetics (Crow, 1987). Its principal task is to relate knowledge of molecular determinants to the phenotype of the intact organism and hence to fitness at the population level. Physiological genetics has yet to undergo the kinds of major advances that have been well documented for both molecular and population genetics.

† To whom correspondence should be addressed.

‡ Present address: Biostatistics, Faculty of Medicine, University of Barcelona, Av. Diagonal s/n. 08028 Barcelona, Spain.

0022-5193/89/210093+22 $03.00/0

It has been clear for some time that new methods are needed to integrate the molecular data into a manageable understanding of the intact system. We have developed a broad theoretic framework, based upon an underlying Power-Law Formalism, in order to address this issue (e.g. Savageau, 1969*b*, 1971*a*, 1972, 1976) and, more specifically, to predict cellular and organismal responses to change in environmental conditions and underlying molecular determinants (Savageau, 1971*a*, 1976, 1979*a,b*, 1980, 1985; Okamoto & Savageau, 1984; Irvine & Savageau, 1985*a,b*; Sorribas & Savageau, 1989*a,b,c*) and to elucidate the design principles of biological systems (Savageau, 1972, 1976, 1979*a,b*, 1980, 1985; Savageau & Jacknow, 1979; Irvine & Saveageau, 1985*a,b*). One of the fundamental features of this non-linear theory is its mathematical structure, which leads to linear algebraic equations in steady state. The relationship between systematic behavior and the underlying molecular parameters is then determined completely by inverting the matrix of coefficients that characterize the system. The orthogonality relationships that characterize the matrix and its inverse also can be interpreted as constraint relationships among the molecular and systemic properties of the system (Savageau, 1971*a*).

Although it has not been generally recognized, others have described an alternative approach to the same theoretical domain that focuses on a set of constraint relationships (e.g. Kacser & Burns, 1973; Heinrich & Rapoport, 1974; Kacser & Burns, 1979; Westerhoff & Chen, 1984; Fell & Sauro, 1985, 1986; Hofmeyr *et al.*, 1986; Kacser & Porteous, 1987) that are equivalent to the orthogonality relationships under special conditions. This approach utilizes two types of measurements that have been given a well-defined meaning and relationship within the context of Biochemical Systems Theory (see Sorribas & Savageau, 1989*a,b*). One they call the "elasticity"; it involves the relative change in the rate of a process resulting from a 1% change in a metabolite concentration that affects that process, while all other metabolite concentrations are held constaint—this is the conventional *kinetic order* of chemical and biochemical kinetics. The second they call the "control coefficient"; it involves the relative change in a flux resulting from a 1% change in an enzyme level or activity, while all other enzyme levels and activities are held constant—this is a special case of the conventional *parameter sensitivity* of systems analysis when enzyme levels and activities are considered parameters of the system.

In this approach, the two types of coefficients—elasticity and control coefficients—are related by various mathematical constraints called summation and connectivity relationships or theorems. The connectivity relationships involve products of elasticities and control coefficients summed over all enzymes in the system; they are critical for expressing control coefficients in terms of elasticities. However, it is the summation relationships, which involve only sums of control coefficients with respect to each of the enzymes in the system, that have received the most attention. This is because the summation is considered to represent a sort of "conservation law" for the influences exerted by the enzymes. Although the distribution of these influences on a given flux can change as conditions vary, the sum of all the influences is conserved and must remain a total of one according to this approach. This is commonly interpreted to mean that if the influence exerted by one enzyme increases then the influence exerted by some other enzyme must correspondingly decrease (e.g. Hartl *et al.*, 1986).

The flux summation relationship has been used to argue that in a system with many enzymes the influence that any given enzyme has on any given flux, as measured by the appropriate control coefficient, must be small (on the average $1/n$ where n is the number of enzymes in the system). Mutations effectively change the amount of an enzyme, and since such changes have only a small effect on any given flux, the consequences of the mutation go unnoticed. Mutant phenotypes will be recessive; the wild type will be dominant. Hence, the sumation relationship alone is sufficient to account for the phenomenon of dominance in genetics (Kacser & Burns, 1981). Others have explored the possibility that this approach might offer means of addressing questions in quantitative genetics (Watt, 1985; Hartl *et al.*, 1985, 1986). Although some have criticized specific uses of this approach (e.g. Stoner, 1984; Burton & Place, 1986; Cornish-Bowden, 1987), and others have made more general criticisms (e.g. Crabtree & Newsholme, 1985, 1987; Hess & Markas, 1987; Savageau, 1987; Sorribas, 1987; Voit, 1987; Welch & Keleti, 1987), until recently this contraint approach has been neither compared with other approaches to the theory nor critically tested.

In order to put in perspective the role of steady state constraints in characterizing and understanding biochemical systems we shall first outline the general theory upon which they are based, and then present the complete set of constraint relationships that follow from first principles within this theory. The results permit a critical comparison of approaches based on constraint relationships with those based directly on the explicit solution of the equations that characterize the system in the Power-Law Formalism. From these comparisons it is seen that: (1) the summation theorem suffers from a number of fundamental limitations that make it invalid for analyzing many if not most realistic biological systems, (2) the more general constraint relationships, while valid, are unable to capture essential information needed to characterize the steady state behavior of such systems, and (3) the explicit solution of the underlying equations allows characterization of dynamic as well as steady state behavior. Thus, approaches based on the underlying equations of the system are superior to those based upon constraint relationships as a foundation for the development of physiological genetics.

2. Biochemical Systems Theory: Mathematical Representation and Explicit Steady State Solution

The Power-Law Formalism refers to a formal mathematical structure that has been systematically elaborated over the past 20 years. It makes frequent use of the common power-law function, which has been used in biology since the time of Galileo, but contains much more. Like other formal languages, e.g. the Linear Formalism, it involves precise definitions, systematic notation, strategies for representation, determination of accuracy, estimation of parameter values, existence theorems, methods of analytical solution and computer analysis, etc. It also provides a canonical non-linear form into which rather arbitrary non-linear functions can be recast exactly. For a recent review, see Savageau & Voit (1987).

The use of the Power-Law Formalism to develop a systematic approach for understanding integrated biochemical systems has led to a general theoretical

framework called Biochemical Systems Theory or BST. This theory can be represented in a number of forms, including the explicit S-system version, which is the case emphasized by Savageau and colleagues, the explicit Generalized Mass Action (GMA) variant, which includes as a special case the Crabtree-Newsholme approach, and the implicit GMA variant, which includes as a special case the approach of Kacser-Burns and Heinrich-Rapoport. (For a detailed treatment of the relatedness of all these variants see Sorribas & Savageau, 1989*a*,*b*,*c*.) Only those elements of BST essential for the purposes of this paper are outlined here; however, sufficient references will be given so that the critical reader can find additional information.

(2.1) S-SYSTEM REPRESENTATION

The fundamental equations in BST are the conservation equations written for each dependent variable in the system, X_i, $i = 1, \ldots, n$†. In the S-system representation, the most useful and accurate of the various representations (Voit & Savageau, 1987; Sorribas & Savageau, 1989*a*,*b*), individual rate laws are first aggregated. Those that characterize reactions forming an X_i are aggregated to give a single rate law for net synthesis, V_i. Similarly, individual rate laws that characterize reactions removing an X_i are aggregated to give a single rate law for net degradation, V_{-i}. Each of these net rate laws for synthesis and degradation is then represented by a product of power-law functions, one for each variable that has an influence upon the net rate law in question. (Step-by-step procedures for constructing the equations can be found in Savageau, 1969*b*, 1976: chapter 9; Voit & Savageau, 1982.) The fundamental equations governing the behavior of the intact biochemical system are then written in BST as

$$dX_i/dt = \alpha_i \prod_{j=1}^{n+m} X_j^{g_{ij}} - \beta_i \prod_{j=1}^{n+m} X_j^{h_{ij}}, \qquad i = 1, \ldots, n \tag{1}$$

where n is the number of dependent concentration variables, m is the number of independent concentration variables, α_i and β_i are the rate constants, and g_{ij} and h_{ij} are the kinetic orders of biochemical kinetics. The parameters α_i and g_{ij} are associated with the rate law for net synthesis of X_i, while β_i and h_{ij} are associated with the rate law for net degradation of X_i.

Algebraic dependencies among the X_i also can be expressed readily in the Power-Law Formalism (Savageau, 1969*b*, 1976, 1979*b*; Savageau *et al.*, 1987*a*). When the redundant equations in (1) are eliminated, and the algebraic dependencies substituted into the remaining equations, the resulting set has exactly the same form as the original set in eqn (1), but with fewer variables. Once the algebraic dependencies have been taken into account in this manner, the representation and subsequent analysis are identical to that given below. (For examples, see Savageau, 1979*b*; Sorribas & Savageau, 1989*a*,*b*.)

† Although the X_i may represent any variable in the system, for simplicity in the presentation we shall henceforth refer to them as biochemical concentration variables. The representation of other types of variables is treated in detail elsewhere (e.g. see Savageau, 1979*b*; Voit & Savageau, 1982; Irvine & Savageau, 1985*a*; Savageau & Voit, 1987; Sorribas & Savageau, 1989*a*).

There are efficient computer methods to examine the dynamic behavior of biochemical systems (Irvine & Savageau, in press; Voit *et al.* 1989), and these have been used in a variety of BST applications (briefly reviewed in Sorribas & Savageau, 1989*a*). However, for the purposes of this paper we need only be concerned with steady state behavior. Thus, we shall say nothing further about dynamic behavior; the interested reader can consult the references given above.

(2.2) STEADY STATE EQUATIONS

In a steady state, the time derivatives are set equal to zero, the resulting non-linear algebraic equations are transformed into a set of linear algebraic equations by taking logarithms, and these are written in conventional matrix notation (Savageau, 1969*b*)

$$[\mathbf{A}]\mathbf{y}] = \mathbf{b}] \tag{2}$$

where the elements of the $(n+m)\times n$ matrix of kinetic orders are given by $a_{ij} = g_{ij} - h_{ij}$, $y_j = \log X_j$, and $b_i = \log(\beta_i/\alpha_i)$. Separation of independent and dependent variables allows the steady state equations to be written as

$$[\mathbf{A}]_d\mathbf{y}]_d = -[\mathbf{A}]_i\mathbf{y}]_i + \mathbf{b}] \tag{3}$$

where the subscript "d" signifies that the matrix $[\mathbf{A}]_d$ contains only kinetic orders with respect to dependent concentrations and the vector $\mathbf{y}]_d$ contains only logarithms of dependent variables. The subscript "i" has the same interpretation but for the independent variables.

(2.3) THE INVERSE OPERATOR

The steady state equations for the S-system of eqn (1) are governed by conventional linear algebra in the logarithms of the dependent variables eqn (3). The matrix of kinetic orders, $[\mathbf{A}]_d$, is always a square matrix of dimension $n \times n$. A fundamental axiom of linear algebra states that for all matrices $[\mathbf{A}]_d$ with non-zero determinant there exists an inverse operator, $[\mathbf{A}]_d^{-1}$, defined as

$$[\mathbf{A}]_d^{-1}[\mathbf{A}]_d = [\mathbf{I}] \tag{4}$$

where $[\mathbf{I}]$ is the $n \times n$ identity matrix (e.g. Bellman, 1960). In order to simplify the notation, we let the inverse operator $[\mathbf{A}]_d^{-1} = [\mathbf{M}]$ (Savageau, 1971*a*). As a consequence of the definition of this operator, it follows that $[\mathbf{A}]_d$ also will be the inverse operator of $[\mathbf{M}]$. That is

$$[\mathbf{M}][\mathbf{A}]_d = [\mathbf{I}] = [\mathbf{A}]_d[\mathbf{M}]. \tag{5}$$

Hence, the inverse operator $[\mathbf{M}]$ is a matrix whose row and column vectors are *orthogonal* to the column and row vectors of $[\mathbf{A}]_d$, respectively. This can be interpreted as

$$\sum_{j=1}^{n} M_{ij}a_{jk} = \delta_{ik}, \qquad i, k = 1, \ldots, n \tag{6}$$

or, since the multiplication of these elements commutes,

$$\sum_{j=1}^{n} a_{ij}M_{jk} = \delta_{ik}, \qquad i, k = 1, \ldots, n \tag{7}$$

where δ_{ik} is the Kronecker delta equal to 1 when $i = k$ and 0 when $i \neq k$.

This orthogonality among the elements of $[\mathbf{A}]_d$ and its inverse operator $[\mathbf{M}]$ is the fundamental basis for the constraint relationships between the different molecular and systemic properties of the system (Savageau *et al.*, 1987*b*). More important, the inverse operator allows one to solve eqn (3) and obtain the dependent variables *explicitly* in terms of the independent variables and parameters of the system (Savageau, 1969*b*).

(2.4) EXPLICIT STEADY STATE SOLUTION

If the conditions for the existence of the inverse operator of the system matrix $[\mathbf{A}]_d$ are fulfilled, the explicit steady state solution can be obtained by premultiplying eqn (3) by $[\mathbf{M}]$. The explicit steady state solution for the S-system in eqn (1) then can be written (Savageau, 1971*a*, 1976)

$$\mathbf{y}]_d = -[\mathbf{M}][\mathbf{A}]_i\mathbf{y}]_i + [\mathbf{M}]\mathbf{b}] = [\mathbf{L}]\mathbf{y}]_i + [\mathbf{M}]\mathbf{b}] \tag{8}$$

where $[\mathbf{L}]$ is a matrix of logarithmic gains (see next section). In this equation the solution for the logarithms of the dependent concentrations $\mathbf{y}]_d$ $(y_j, j = 1, \ldots, n)$ is divided into two parts. The first exhibits the linear dependence on the logarithms of the independent concentrations $\mathbf{y}]_i$ $(y_j, j = n+1, \ldots, n+m)$; the second exhibits the linear dependence on the logarithms of the rate constants $\mathbf{b}]$ $[b_i = \log(\beta_i/\alpha_i), i = 1, \ldots, n]$.

The flux through any pool X_i in steady state is obtained by a simple secondary calculation involving the known concentrations in steady state.

$$(\log \mathbf{V}_+)] = (\log \boldsymbol{\alpha})] + [\mathbf{G}]\mathbf{y}] \quad \text{or} \quad (\log \mathbf{V}_-)] = (\log \boldsymbol{\beta})] + [\mathbf{H}]\mathbf{y}] \tag{9}$$

where $(\log \mathbf{V}_+)]$, $(\log \mathbf{V}_-)]$, $(\log \boldsymbol{\alpha})]$, and $(\log \boldsymbol{\beta})]$ are n vectors representing the logarithms of influxes $(\log V_i, i = 1, \ldots, n)$ effluxes $(\log V_{-i}, i = 1, \ldots, n)$, α rate constants $(\log \alpha_i, i = 1, \ldots, n)$, and β rate constants $(\log \beta_i, i = 1, \ldots, n)$. The elements of the $(n+m) \times n$ matrices $[\mathbf{G}]$ and $[\mathbf{H}]$ are given by the kinetic orders g_{ij} and h_{ij}, respectively. Thus, the explicit solution in eqns (8) and (9) gives the complete relationship in BST between the steady state values of the dependent variables on the one hand and the values of the independent variables and parameters of the system on the other.

3. Factors Relating Systemic Behavior to the Underlying Kinetic-Order Parameters

The behavior of a complex biochemical system is characterized by the responses of the dependent variables to changes in the independent variables and parameters of the system. The explicit solution obtained with the S-system representation provides a complete characterization of the local steady state behavior about any

operating point of a biochemical system (Savageau, 1969*b*, 1972; Savageau *et al.*, 1987*a,b*; Sorribas & Savageau, 1989*a,b,c*). However, it also is useful to characterize the system in terms of standard factors that relate its global properties to its underlying molecular determinants; namely logarithmic gains and parameter sensitivities (Savageau, 1971*a*; Sorribas & Savageau, 1989*a,b,c*).

(3.1) CONCENTRATION BEHAVIOR

The systemic behavior of the dependent concentrations is given explicitly by eqn (8). The factors that characterize the systemic response to change in specific independent concentrations or parameters can be readily determined by appropriate differentiation of the explicit solution.

Logarithmic gains

The percentage change in a dependent concentration X_i resulting from a 1% increase in an independent concentration X_k, while all other independent concentrations and parameters are held constant, can be determined by differentiation of eqn (8).

$$\begin{aligned} \partial y_i/\partial y_k &= (\partial X_i/\partial X_k)(X_k/X_i) = L_{ik} \\ &= L(X_i, X_k), \qquad i = 1, \ldots, n;\ k = n+1, \ldots, n+m. \end{aligned} \tag{10}$$

Hence, the elements L_{ik} of the $m \times n$ matrix $[\mathbf{L}] = -[M][\mathbf{A}]_i$ are analogous to gain or amplification factors in conventional network theories (e.g. Bode, 1945) and within BST they are referred to as *logarithmic gain* factors (Savageau, 1971*a*, 1972, 1976; Savageau *et al.*, 1987*b*; Sorribas & Savageau, 1989*a*). The matrix $[\mathbf{L}]$ is also represented by the symbol $[\mathbf{L}(X, X)]$.

Rate-constant sensitivities

The percentage change in a dependent concentration X_i resulting from a 1% increase in a rate constant β_j or a 1% decrease in a rate constant α_j can be determined by differentiation of the explicit solution with respect to the parameter b_j.

$$\begin{aligned} \partial y_i/\partial b_j &= (\partial X_i/\partial b_j)(b_j/X_i) = M_{ij} \\ &= S(X_i, \beta_j) = -S(X_i, \alpha_j), \qquad i, j = 1, \ldots, n. \end{aligned} \tag{11}$$

Hence, the elements M_{ij} of the $n \times n$ matrix $[\mathbf{M}]$, which is the inverse of the $n \times n$ system matrix $[\mathbf{A}]_d$, are identical to conventional parameter sensitivities (Bode, 1945; Cruz, 1973; Savageau, 1971*a,b*, 1976; Savageau *et al.*, 1987*b*). In BST they are referred to as *rate-constant sensitivites.* The matrix $[\mathbf{M}]$ is also represented by the symbols $[\mathbf{S}(X, \beta)] = -[\mathbf{S}(X, \alpha)]$.

Kinetic-order sensitivities

In a similar fashion, one can differentiate the explicit solution in eqn (8) with respect to one of the kinetic orders in the system and thereby determine the percentage change in a dependent concentration X_i resulting from a one-percent change in a

kinetic order g_{kp} or h_{kp}. These factors are defined as *kinetic-order sensitivities*, and they are related in several different ways (Savageau, 1971*a*; Savageau *et al.*, 1987*b*; Sorribas & Savageau, 1989*a*).

$$(\partial X_i/\partial g_{kp})(g_{kp}/X_i) = S(X_i, g_{kp}) = y_i S(y_i, g_{kp})$$

$$\left(\frac{S(X_i, g_{kp})}{g_{kp}}\right) = -\left(\frac{S(X_i, h_{kp})}{h_{kp}}\right)$$

$$\left(\frac{S(y_i, g_{kp})}{g_{kp}}\right) = -\left(\frac{S(y_i, h_{kp})}{h_{kp}}\right).$$

By analogy to the identification of the elements M_{ij}, with the rate-constant sensitivities, one can identify the elements

$$N_{ikp} = \left(\frac{S(X_i, h_{kp})}{h_{kp}}\right) = -\left(\frac{S(X_i, g_{kp})}{g_{kp}}\right), \qquad i, k = 1, \ldots, n; \; p = 1, \ldots, n+m \quad (12)$$

of the three-dimensional tensor $\{\mathbf{N}\}$ with the kinetic-order sensitivities. The tensor $\{\mathbf{N}\}$ is also represented by the symbols $\{\mathbf{S}(X, h)/h\} = -\{\mathbf{S}(X, g)/g\}$.

(3.2) FLUX BEHAVIOR

The systemic behavior of the dependent fluxes is given by a simple secondary calculation involving the known concentrations in steady state [eqn (9)]. The factors that characterize the systemic response to change in specific independent concentrations or parameters can be determined readily by appropriate differentiation and use of the results given above.

Logarithmic gains

The effect of change in an independent variable on a dependent flux can be determined by differentiation of eqn (9). Hence, the logarithmic gains for the fluxes are

$$L(V_i, X_k) = g_{ik} + \sum_{j=1}^{n} g_{ij} L(X_j, X_k), \qquad i = 1, \ldots, n; \; k = n+1, \ldots, n+m$$

or

$$[\mathbf{L}(V_+, X)] = [\mathbf{G}]_i + [\mathbf{G}]_d [\mathbf{L}(X, X)] \quad (13)$$

where the subscripts "i" and "d" signify that the matrix contains only kinetic orders with respect to the independent or dependent variables, respectively.

Rate-constant sensitivities

By a similar procedure, one obtains the following rate-constant sensitivities

$$S(V_i, \alpha_k) = \delta_{ik} + \sum_{j=1}^{n} g_{ij} S(X_j, \alpha_k), \qquad i, k = 1, \ldots, n$$

or

$$[\mathbf{S}(V_+, \alpha)] = [\mathbf{I}] + [\mathbf{G}]_d [\mathbf{S}(X, \alpha)] \quad (14)$$

and

$$S(V_i, \beta_k) = \sum_{j=1}^{n} g_{ij} S(X_j, \beta_k), \qquad i, k = 1, \ldots, n$$

or

$$[\mathbf{S}(V_+, \beta)] = [\mathbf{G}]_d [\mathbf{S}(X, \beta)]. \tag{15}$$

Kinetic-order sensitivities

Similarly, the kinetic-order sensitivities are

$$\left(\frac{S(V_i, g_{kp})}{g_{kp}}\right) = \delta_{ik} y_p + \sum_{j=1}^{n} g_{ij} \left(\frac{S(X_j, g_{kp})}{g_{kp}}\right), \qquad i, k = 1, \ldots, n; \; p = 1, \ldots, n+m$$

or

$$\{\mathbf{S}(V_+, g/g\} = [\mathbf{I}] \otimes \mathbf{y}] + [\mathbf{G}]_d \otimes \{\mathbf{S}(X, g)/g\} \tag{16}$$

and

$$\left(\frac{S(V_i, h_{kp})}{h_{kp}}\right) = \sum_{j=1}^{n} g_{ij} \left(\frac{S(X_j, h_{kp})}{h_{kp}}\right), \qquad i, k = 1, \ldots, n; \; p = 1, \ldots, n+m$$

or

$$\{\mathbf{S}(V_+, h)/h\} = [\mathbf{G}]_d \otimes \{\mathbf{S}(X, h)/h\} \tag{17}$$

where the symbol $\otimes$ indicates (matrix) multiplication of the left-hand matrix with the pth matrix of the right-hand tensor, or (scalar) multiplication of the left-hand matrix with the pth element of the right-hand vector, to generate the pth matrix of the resulting three-dimensional tensor.

By means of the factors described in this section one can characterize the systemic response to change in each independent concentration and each parameter of the system, and, because these factors can be expressed explicitly in terms of the kinetic orders of the system, they also are important for relating systemic behavior to the underlying determinants of the system (Savageau, 1971*a*; Sorribas & Savegeau, 1989*a*,*b*). These relationships are summarized in Table 1.

TABLE 1

Complete Characterization of the Nominal Steady State within BST

Flux Variables		Concentration Variables	
Systemic Properties	Component Properties	Systemic Properties	Component Properties
$[\mathbf{L}(V, X)] = [\mathbf{G}]_i - [\mathbf{G}]_d [\mathbf{A}]_d^{-1} [\mathbf{A}]_i$		$[\mathbf{L}(X, X)] = -[\mathbf{A}]_d^{-1} [\mathbf{A}]_i$	
$[\mathbf{S}(V, \beta)] = [\mathbf{G}]_d [\mathbf{A}]_d^{-1}$		$[\mathbf{S}(X, \beta)] = [\mathbf{A}]_d^{-1}$	
$[\mathbf{S}(V, \alpha)] = [\mathbf{I}] - [\mathbf{G}]_d [\mathbf{A}]_d^{-1}$		$[\mathbf{S}(X, \alpha)] = -[\mathbf{A}]_d^{-1}$	
$\{\mathbf{S}(V, h)/h\} = [\mathbf{G}]_d [\mathbf{A}]_d^{-1} \otimes \mathbf{y}]$		$\{\mathbf{S}(X, h)/h\} = [\mathbf{A}]_d^{-1} \otimes \mathbf{y}]$	
$\{\mathbf{S}(V, g)/g\} = [\mathbf{I}] \otimes \mathbf{y}] - [\mathbf{G}]_d [\mathbf{A}]_d^{-1} \otimes \mathbf{y}]$		$\{\mathbf{S}(X, g)/g\} = -[\mathbf{A}]_d^{-1} \otimes \mathbf{y}]$	

4. Orthogonality Properties

The existence of an inverse operator defined by the relationship $[\mathbf{A}]_d[\mathbf{M}] = [\mathbf{I}]$, which is an axiomatic property of linear systems, can be interpreted alternatively as a set of orthogonality properties involving kinetic orders (molecular determinants) and parameter sensitivities (systemic properties). This orthogonality is inherent to the linear structure of the steady state equations in the S-system representation of BST, and the specific biochemical interpretation is the result of identifying the elements of the inverse operator with the rate-constant sensitivities of the system [eqn (10)]. One might ask, does the linear structure of the steady state equations imply a similar orthogonality for the other systemic properties? The answer is yes, as can be seen by appropriate differentiation of the steady state equations. These additional manifestations of the orthogonality properties are presented below.

(4.1) MANIFESTATIONS INVOLVING LOGARITHMIC GAINS

Logarithmic differentiation of eqn (3) with respect to an independent variable X_k yields the set of equations

$$\sum_{j=1}^{n} a_{ij}L_{jk} = -a_{ik}, \qquad i = 1, \ldots, n;\ k = n+1, \ldots, n+m$$

or

$$[\mathbf{A}]_d[\mathbf{L}] = -[\mathbf{A}]_i. \tag{18}$$

This set of equations, involving logarithmic gains and kinetic orders, is formally equivalent to the fundamental orthogonality properties expressed by eqn (5). According to the definition of the matrix $[\mathbf{L}]$, eqn (18) can be rewritten $-[\mathbf{A}]_d[\mathbf{M}][\mathbf{A}]_i = -[\mathbf{A}_i]$, which is equivalent to $[\mathbf{A}]_d[\mathbf{M}] = [\mathbf{I}]$.

(4.2) MANIFESTATIONS INVOLVING RATE-CONSTANT SENSITIVITIES

Logarithmic differentiation of eqn (3) with respect to rate-constant parameters α_k or β_k yields

$$\sum_{j=1}^{n} a_{ij}M_{jk} = \delta_{ik}, \qquad i, k = 1, \ldots, n$$

or

$$[\mathbf{A}]_d[\mathbf{M}] = [\mathbf{I}] \tag{19}$$

and, since this matrix multiplication commutes,

$$[\mathbf{M}][\mathbf{A}]_d = [\mathbf{I}]. \tag{20}$$

These equations are the fundamental orthogonality properties previously identified with the axiomatic definition of the inverse operator in linear algebra [eqn (5)].

(4.3) MANIFESTATIONS INVOLVING KINETIC-ORDER SENSITIVITIES

Similarly, logarithmic differentiation with respect to kinetic-order parameters g_{kp} or h_{kp} yields

$$\sum_{j=1}^{n} a_{ij} N_{jkp} = \delta_{ik} y_p, \qquad i, k = 1, \ldots, n; \; p = 1, \ldots, n+m$$

or

$$[\mathbf{A}]_d \otimes \{\mathbf{N}\} = [\mathbf{I}] \otimes \mathbf{y}] \tag{21}$$

and, since the matrix multiplications commute,

$$\{\mathbf{N}\} \otimes [\mathbf{A}]_d = [\mathbf{I}] \otimes \mathbf{y}]. \tag{22}$$

By comparing eqns (19) and (21), one can see that

$$\{\mathbf{N}\} = [\mathbf{M}] \otimes \mathbf{y}]. \tag{23}$$

From this last result it follows that eqns (21) and (22) are equivalent to eqns (5). As in the case of logarithmic gains and rate-constant sensitivities, the equations relating kinetic-order sensitivities and kinetic orders are but an alternative expression of the orthogonality properties associated with the existence of the inverse operator of the system matrix $[\mathbf{A}]_d$.

The orthogonality properties in this section are automatically incorporated into the explicit steady state solution. Hence, they can not be used to obtain any additional information beyond that available from the steady state solution.

5. Complete Set of Constraint Relationships

The complete steady state characterization of a biochemical system in BST consists of determining *explicitly* all the dependent concentration variables and fluxes in steady state, and how these are influenced by all the parameters and independent variables of the system (Savageau, 1969*b*, 1971*a*, 1972). The explicit steady state solution in BST is both necessary and sufficient for this characterization.

In contrast, the specific constraint relationships of Kacser *et al.*—the summation and connectivity theorems—are neither necessary nor sufficient for characterizing intact biochemical systems (Savageau *et al.*, 1987*a*,*b*; Sorribas & Savageau, 1989*a*,*b*,*c*). Nonetheless, it has been argued that the summation theorem can be used to place constraints upon the values of these systemic coefficients (Kacser & Burns, 1979).

Since BST allows a complete characterization of the system, it is possible to put in proper perspective the role of such constraint relationships. In fact, a number of more general constraint relationships in the Power-Law Formalism already have been demonstrated to include the specific summation and connectivity relationships proposed by Kacser and his colleagues (Savageau *et al.*, 1987*b*). These more general constraint relationships are mathematically equivalent to the well-known orthogonality properties of linear systems (see the previous section). They are true by definition and so cannot be "proved" because they are part of the axiomatic structure of linear

algebra. Although these constraint relationships yield no information not already provided by the explicit solution in BST, they might offer another view on the relationship among the various elements of the system characterization.

There are potentially 24 constraint relationships in the full set, which is generated by combination of the following options in BST: 3 (rate-constant parameters, kinetic-order parameters, independent variables) × 2 (dependent concentrations, dependent fluxes) × 2 (summation, connectivity) × 2 (direct orthogonality properties, commuted orthogonality properties). Of these, 15 correspond to readily interpretable constraints, while the remaining nine cannot be realized or have no obvious meaning. The two different forms of the orthogonality properties may be interpreted as follows. In one case, a simultaneous 1% change is made in each of the parameters in a set. The orthogonality property then gives the "partitioning" of the single net effect on a given dependent variable of the system into component effects that can be attributed to each of the parameters. In the other, a 1% change is made in a single parameter pair or independent variable. The orthogonality property then gives the "distribution" of the multiple effects that are spread over the dependent variables of the system. It is important to note that no conservation of total influence is implied in either case.

(5.1) PARTITIONING OF EFFECTS AMONG PARAMETERS

For this set of constraint relationships one considers responses in a single dependent concentration variable X_i, or a single dependent flux variable V_i, that result from changes in an entire class of parameters. The parameter sensitivities measure the response to change in the individual parameters while all other parameters are held constant. Alternatively, one might consider a simultaneous 1% change in all the parameters of the class, and then the parameter sensitivities measure the contribution to the net change that can be attributed to each parameter.

Summation of rate-constant effects on concentrations

Consider a given dependent concentration variable X_i, determine the parameter sensitivity with respect to each of the rate constants in the system, sum these, and note the relationships in eqn (11). The result is

$$\sum_{j=1}^{n} [S(X_i, \alpha_j) + S(X_i, \beta_j)] = 0, \qquad i = 1, \ldots, n. \tag{24}$$

That is, for each dependent concentration, the sum of the sensitivities with respect to change in the rate-constant parameters is zero.

Connectivity of rate-constant effects on concentrations

If one notes $a_{jk} = g_{jk} - h_{jk}$ [see eqn (2)] and the relationships in eqn (11), then it is clear that the orthogonality properties in eqn (6) can be rewritten as

$$\sum_{j=1}^{n} [S(X_i, \alpha_j) g_{jk} + S(X_i, \beta_j) h_{jk}] = -\delta_{ik}, \qquad i, k = 1, \ldots, n. \tag{25}$$

For each dependent concentration, the sum of the sensitivities with respect to change in the rate-constant parameters multiplied by the associated kinetic order with respect to a second dependent concentration X_k is -1 if the first and the second concentration are the same or 0 if they are different.

Summation of rate-constant effects on fluxes

Consider a given dependent flux variable V_i, determine the sensitivities from eqns (14) and (15), sum these, and note the relationships in eqn (11). The result is

$$\sum_{j=1}^{n} [S(V_i, \alpha_j) + S(V_i, \beta_j)] = 1, \qquad i = 1, \ldots, n. \tag{26}$$

For the flux through each dependent pool, the sum of the sensitivities with respect to change in the rate-constant parameters is unity. Equations (14), (15) and (11) also imply

$$[\mathbf{S}(V_+, \alpha)] = [\mathbf{H}]_d[\mathbf{S}(X, \alpha)]$$

$$[\mathbf{S}(V_+, \beta)] = [\mathbf{G}]_d[\mathbf{S}(X, \beta)]$$

from which it follows that eqn (26) can be rewritten as

$$[\mathbf{H}]_d[\mathbf{S}(X, \alpha)] + [\mathbf{G}]_d[\mathbf{S}(X, \beta)] = [\mathbf{A}]_d[\mathbf{M}] = [\mathbf{I}].$$

Connectivity of rate-constant effects on fluxes

If one determines the sensitivities from eqns (14) and (15), and notes the relationships in eqn (11) and the fact that $a_{jk} = g_{jk} - h_{jk}$ [see eqn (2)], then the orthogonality properties in eqn (6) can be rewritten as

$$\sum_{j=1}^{n} [S(V_i, \alpha_j) g_{jk} + S(V_i, \beta_j) h_{jk}] = 0, \qquad i, k = 1, \ldots, n. \tag{27}$$

For the flux through each dependent pool X_i, the sum of the sensitivities with respect to change in the rate-constant parameters multiplied by the associated kinetic order with respect to a second dependent concentration X_k is zero.

Summation of kinetic-order effects on concentrations

Consider a given dependent concentration variable X_i, determine its parameter sensitivities with respect to each of the kinetic orders in the system that involves a second concentration variable X_p, sum these, and note the relationships in eqn (12). The result is

$$\sum_{j=1}^{n} \left\{ \left(\frac{S(X_i, g_{jp})}{g_{jp}} \right) + \left(\frac{S(X_i, h_{jp})}{h_{jp}} \right) \right\} = 0, \qquad i = 1, \ldots, n; \; p = 1, \ldots, n+m. \tag{28}$$

For each dependent concentration, the sum of the sensitivities with respect to change in each of the kinetic-order parameters that involves a second concentration variable, when divided by the corresponding kinetic order, is zero.

Connectivity of kinetic-order effects on concentrations

If one notes $a_{jk} = g_{jk} - h_{jk}$ [see eqn (2)] and the relationships in eqn (12), then it is clear that the orthogonality properties in eqn (22) can be rewritten as

$$\sum_{j=1}^{n} \left\{ \left(\frac{S(X_i, g_{jp})}{g_{jp}} \right) g_{jk} + \left(\frac{S(X_i, h_{jp})}{h_{jp}} \right) h_{jk} \right\} = -\delta_{ik} y_p,$$
$$i, k = 1, \ldots, n; \; p = 1, \ldots, n+m. \tag{29}$$

For each dependent concentration X_i, the sum of the sensitivities with respect to change in each of the kinetic orders that involves a second concentration variable X_p, when divided by the corresponding kinetic order and multiplied by the associated kinetic order with respect to another dependent concentration X_k, is $-\log X_p$ if the first and the last dependent concentrations are the same or 0 if they are different.

Summation of kinetic-order effects on fluxes

Consider a given dependent flux variable V_i, determine the sensitivities from eqns (16) and (17), sum these, and note the relationships in eqn (12). The result is

$$\sum_{j=1}^{n} \left\{ \left(\frac{S(V_i, g_{jp})}{g_{jp}} \right) + \left(\frac{S(V_i, h_{jp})}{h_{jp}} \right) \right\} = y_p, \qquad i = 1, \ldots, n; \; p = 1, \ldots, n+m. \tag{30}$$

For the flux through each dependent pool X_i, the sum of the sensitivities with respect to change in each of the kinetic-order parameters that involves a second concentration X_p divided by the corresponding kinetic orders is equal to $\log X_p$.

Connectivity of kinetic-order effects on fluxes

If one determines the sensitivities from eqns (16) and (17), and notes the relationships in eqn (12) and the fact that $a_{jk} = g_{jk} - h_{jk}$ [see eqn (2)], then the orthogonality properties in eqn (22) can be rewritten as

$$\sum_{j=1}^{n} \left\{ \left(\frac{S(V_i, g_{jp})}{g_{jp}} \right) g_{jk} + \left(\frac{S(V_i, h_{jp})}{h_{jp}} \right) h_{jk} \right\} = 0, \qquad i, k = 1, \ldots, n; \; p = 1, \ldots, n+m. \tag{31}$$

For the flux through each dependent pool X_i, the sum of the sensitivities with respect to change in each of the kinetic-order parameters that involves a second concentration X_p, when divided by the corresponding kinetic order and multiplied by the associated kinetic order with respect to another dependent concentration X_k, is zero.

Summary

The first four constraint relationships, involving rate-constant parameters, have been given elsewhere (Savageau *et al.*, 1987*b*). The second four, involving kinetic-order parameters, are new. There are four more constraint relationships that might be considered in this class. The logarithmic gains in dependent concentrations summed over all the independent concentrations, and the logarithmic gains in dependent fluxes summed over all the independent concentrations,

$$\sum_{j=n+1}^{n+m} L(X_i, X_j) \quad \text{and} \quad \sum_{j=n+1}^{n+m} L(V_i, X_j), \qquad i = 1, \ldots, n$$

yield no readily interpreted result. The corresponding connectivity relationships

$$\sum_{j=n+1}^{n+m} L(X_i, X_j)a_{jk} \quad \text{and} \quad \sum_{j=n+1}^{n+m} L(V_i, X_j)a_{jk}, \qquad i=1,\ldots,n;\ k=1,\ldots,n+m$$

do not exist because there are no kinetic orders with first subscripts in the range $j > n$.

(5.2) DISTRIBUTION OF EFFECTS OVER DEPENDENT VARIABLES

The following set of constraint relationships is obtained by simply commuting the orthogonality properties of the system. The responses are determined in each case for a given change in a single parameter pair or independent concentration, and these are summed over all the dependent concentrations or fluxes in the system. The results are to be contrasted with those in the previous section, where the responses involve a single dependent concentration or flux and the sum is over all parameters of a given class.

Summation of rate-constant effects on concentrations

Consider the rate constants for net synthesis and net degradation of a given dependent concentration X_k, determine the sensitivities to change in these parameters for all dependent concentrations in the system, sum these, and note the relationships in eqn (11). The result is

$$\sum_{j=1}^{n} [S(X_j, \alpha_k) + S(X_j, \beta_k)] = 0, \qquad k=1,\ldots,n. \tag{32}$$

For each such rate-constant pair, the sum of the parameter sensitivities over all dependent concentrations in the system is zero.

Connectivity of rate-constant effects on concentrations

If one notes $a_{jk} = g_{jk} - h_{jk}$ [see eqn (2)] and the relationships in eqn (11), then it is clear that the orthogonality properties in eqn (7) can be rewritten as

$$\sum_{j=1}^{n} [g_{ij}S(X_j, \alpha_k) + h_{ij}S(X_j, \beta_k)] = -\delta_{ik}, \qquad i, k=1,\ldots,n. \tag{33}$$

For each rate-constant pair corresponding to the dependent concentration X_k, the sum of the sensitivities multiplied by the kinetic orders for synthesis and degradation of a second dependent concentration X_i is -1 if the first and the second concentration are the same or 0 if they are different.

Summation of rate-constant effects on fluxes

Consider a given rate-constant pair corresponding to the dependent concentration X_k, determine the sensitivities from eqns (14) and (15), sum these, and note the relationships in eqn (11). The result is

$$\sum_{j=1}^{n} [S(V_j, \alpha_k) + S(V_j, \beta_k)] = 1, \qquad k=1,\ldots,n. \tag{34}$$

For each rate-constant pair, the sum of the sensitivities over all dependent fluxes is unity. This relationship is equivalent to the orthogonality properties in eqn (5) as can be shown by the procedure following eqn (26).

Summation of kinetic-order effects on concentrations

Consider the pair of kinetic orders g_{kp} and h_{kp}, determine the sensitivities of all dependent concentrations in the system with respect to each, sum these, and note the relationships in eqn (12). The result is

$$\sum_{j=1}^{n} \left\{ \left(\frac{S(X_j, g_{kp})}{g_{kp}} \right) + \left(\frac{S(X_j, h_{kp})}{h_{kp}} \right) \right\} = 0, \qquad k = 1, \ldots, n; \; p = 1, \ldots, n+m. \quad (35)$$

For each kinetic-order pair, the sum of the sensitivities for all dependent concentrations divided by the corresponding kinetic order, is zero.

Connectivity of kinetic-order effects on concentrations

If one notes $a_{jk} = g_{jk} - h_{jk}$ [see eqn (2)] and the relationships in eqn (12), then it is clear that the orthogonality properties in eqn (21) can be rewritten as

$$\sum_{j=1}^{n} \left\{ g_{ij} \left(\frac{S(X_j, g_{kp})}{g_{kp}} \right) + h_{ij} \left(\frac{S(X_j, h_{kp})}{h_{kp}} \right) \right\} = -\delta_{ik} y_p,$$
$$i, k = 1, \ldots, n; \; p = 1, \ldots, n+m. \quad (36)$$

For each pair of kinetic orders associated with the dependent concentration X_k, the sum of the sensitivities, when divided by the corresponding kinetic orders and multiplied by the kinetic orders for synthesis and degradation of a second dependent concentration X_i, is $-\log X_p$ if the first and the second concentration are the same or 0 if they are different.

Summation of kinetic-order effects on fluxes

Consider the pair of kinetic orders g_{kp} and h_{kp}, determine the sensitivities from eqns (16) and (17), sum these, and note the relationships in eqn (12). The result is

$$\sum_{j=1}^{n} \left\{ \left(\frac{S(V_j, g_{kp})}{g_{kp}} \right) + \left(\frac{S(V_j, h_{kp})}{h_{kp}} \right) \right\} = y_p, \qquad k = 1, \ldots, n; \; p = 1, \ldots, n+m. \quad (37)$$

For each kinetic-order pair, the sum of the sensitivities for all dependent fluxes divided by the corresponding kinetic order, is $\log X_p$.

Connectivity of independent-variable effects on concentrations

These constraints are simply an alternative manifestation of the orthogonality properties, as already noted for eqn (18).

$$\sum_{j=1}^{n} a_{ij} L(X_j, X_k) = -a_{ik}, \qquad i = 1, \ldots, n; \; k = n+1, \ldots, n+m. \quad (38)$$

For each independent concentration X_k, the sum of the logarithmic gains for all dependent concentrations, multiplied by the corresponding kinetic orders for synthesis and degradation of a given dependent concentrations X_i, is equal to the negative of the kinetic orders with respect to the independent concentration X_k.

Summary

All the constraint relationships in this section are new. There are five more constraint relationships that might be considered in this class, but none of them yields a readily interpreted result.

6. Implications for Physiological Genetics

Having generated the complete set of constraint relationships within the general framework provided by BST, we are now in a position to discuss their role in understanding the integrated behavior of biochemical systems and, hence, their implications for physiological genetics. We shall begin by considering a specific example of how constraint relationships have been used.

(6.1) DOMINANCE

Kacser & Burns (1981) argue that most mutations are recessive (wild type dominant) as a necessary consequence of a summation relationship or theorem that shows how the influences on a given systemic flux are distributed over all the enzymes in the system. In their approach the enzyme levels are considered parameters of the system, and the influence of each enzyme is determined by its sensitivity coefficient. Hence, if one characterizes the system according to this approach, "[t]here are as many sensitivity coefficients for a given flux as there are enzymes in the system. It can be shown that the sum of all such coefficients equals unity." It is claimed that this summation property has been proved for systems of any structural complexity. As a consequence, "[s]ince n, the number of enzymes, is large, this summation property results in the individual coefficients being small." From these considerations Kacser & Burns (1981) suggest that "[t]he widespread occurrence of recessive mutants is thus seen to be the inevitable consequence of the kinetic structure of enzyme networks." This argument, it is claimed, eliminates the necessity to invoke evolution as the origin of the phenomenon. Clearly, this argument stands or falls on the general validity of the summation theorem, which until recently has not been subjected to critical analysis. The results of such analysis show that the summation theorem suffers from at least four major limitations.

(6.2) CRITIQUE OF THE SUMMATION THEOREM

The summation theorem is not valid for systems that involve enzyme-enzyme complexes (Sorribas & Savageau, 1989*a*,*b*). There is now abundant experimental evidence and sound theoretical reasons for the existence of enzyme-enzyme interactions in biochemical systems. The rates in such systems need not be linearly independent functions of enzyme levels, as is assumed in the derivation of the summation theorem (Kacser & Burns, 1973, 1979). Even in cases where the total enzyme concentration is truly an independent variable, e.g. *in vitro* experiments with purified enzymes, its influence on the rate of an individual reaction need not be linearly dependent upon the enzyme level when it interacts with other enzymes. It has been shown clearly for such systems that the sum of the sensitivity coefficients is not equal to unity and that individual empirically determined sensitivities can have values much greater than unity (Sorribas & Savageau, 1989*b*). As this work also shows, the method of direct solution in BST (Savageau, 1971*a*) allows systems involving enzyme-enzyme complexes to be analyzed without difficulty.

The summation theorem is not valid for systems that involve reactions at or very near thermodynamic equilibrium or that pass through equilibrium (Stoner, 1984; Sorribas & Savageau, 1989*c*). Although it is well known that biological organisms operate far from thermodynamic equilibrium, there are subsystems that under certain conditions do function at or very near equilibrium and do pass through equilibrium. Among the most extensively studied are glycolytic reactions that under appropriate conditions reverse their direction of operation to participate in gluconeogenesis. The sensitivity coefficients in the Kacser-Burns approach can not be determined for a flux when that flux is at or very near zero. (Although one can conceive of determining kinetic orders and sensitivities even very near equilibrium, careful analysis shows that such determinations become highly unreliable.) BST provides strategies for dealing with systems that pass through equilibrium (Sorribas & Savageau, 1989*c*).

The summation theorem is not valid for determining the distribution of influence when there are negative sensitivities (Savageau *et al.*, 1987*b*), which is the case for most if not all biological systems. For example, this becomes an important issue for systems that include branched pathways, feedback activation mechanisms, feed-forward inhibition mechanisms, or cascade mechanisms such as enzyme-proenzyme cascades and the transcription-translation cascades of gene expression. These are clearly documented features of most biological systems. If the sensitivities do indeed sum to unity, then there must be other coefficients with positive values that can be greater than unity. There is no longer a "unit amount of influence" that is conserved; the influence of one enzyme can increase and that of the others need not decrease. Clearly, sensitivity coefficients need not be positive fractional quantities, their average magnitude need not be $1/n$, and individual values need not be small. The situation is even clearer in the case of influences on metabolic concentrations, where under corresponding conditions the sum of sensitivity coefficients for all enzymes equals zero. In this case, the positive influences must be balanced by an equivalent amount of negative influence, and in general individual sensitivity coefficients need not be small.

The summation theorem is restricted by its implicit representation of the underlying kinetics and its assumption of a stable steady state. Such constraint relationships do not allow one to characterize fully an actual steady state because they lack one fundamental class of parameters in the underlying formalism, the rate constants; and they are unable to verify the existence or stability of a steady state because the dynamic structure of the systems is missing (Savageau *et al.*, 1987*a*; Sorribas & Savageau, 1989*b*). It is well known that small changes in parameter values or independent variables may not lead to similarly small changes in steady state properties, but may in fact produce large qualitative shifts to another steady state or to another mode of dynamic behavior such as stable oscillation or chaotic fluctuation (e.g. see Hess & Markas, 1987). In order to determine that a steady state analysis is indeed appropriate when there is the possibility of such behaviors requires a theory capable of representing the dynamic structure of the system.

The one severely restricted case where clearly the summation theorem applies and the average sensitivity coefficient is given by $1/n$ is the case of an unbranched pathway of soluble enzymes operating far from equilibrium in a first-order regime

with no enzyme-enzyme interactions and no allosteric regulation. This is the case actually analyzed by Kacser & Burns (1981). Although the essential insights were provided by the earlier analysis of this same case by Waley (1964), what is new in the analysis by Kacser & Burns (1981) is the claim that these conclusions are inevitably true for all systems and that they automatically account for the phenomenon of dominance. The generality of this claim rests upon their assumption that the summation theorem is valid for systems "of any structural complexity", which as noted above is not valid, and upon their arguments that the influence of negative sensitivities can be discounted, which is questionable.

In conclusion, the assumptions that underlie the summation theorem in the Kacser-Burns approach are not generally valid. Even in cases where the assumptions are valid and the sensitivities do indeed sum to unity, the use of this relationship to express a conservation of influence among the enzymes is still invalid for most if not all biological systems. Hence, dominance cannot be explained on the basis of such a conservation principle.

(6.3) CRITIQUE OF THE GENERAL CONSTRAINT RELATIONSHIPS

One response to the above limitations is to search for an appropriate generalization of the summation and connectivity relationships that constitute the fundamental principles of the Kacser-Burns approach. Since it is known that their approach is a special case of Biochemical Systems Theory (e.g. see Savageau *et al.*, 1987*a*,*b*; Sorribas & Savageau, 1989*a*,*b*), an appropriate generalization of the summation relationships is readily available. In fact, as we have seen in this paper, one can exhaust the possibilities for such constraint relationships within BST. The constraint relationships in BST are more general because the full set of fundamental parameters is identified, because the sensitivities are defined with respect to all of the fundamental parameters and not restricted to just enzyme activities, and because linearity and independence of enzyme forms is not assumed. Even though the constraint relationships are mathematically valid they are not always biologically meaningful. Thus, one must ask: what additional information do these generalized constraint relationships provide, can they be used to provide a sound explanation of dominance, and more generally, can they provide a foundation for addressing the larger spectrum of questions that are important in physiological genetics?

The summation relationship of Kacser & Burns is not among the more general constraints. When its restrictions are taken into account, it becomes clear that it is a special case of eqn (26) (see Savageau *et al.*, 1987*b*). It also is clear from an examination of this equation and the other general constraint relationships given in the previous section that individual sensitivities and logarithmic gains need not be small positive quantities. As we have shown elsewhere, the individual sensitivities in specific systems can indeed be quite large (Sorribas & Savageau, 1989*a*,*b*,*c*). Thus, even generalized summation relationships that validly sum to unity, fail as "conservation laws" for the influence upon systemic properties. We can conclude that dominance is not a necessary consequence of the more general constraint relationships.

The broader issue is whether or not an approach based on constraint relationships might provide something new in the way of methodology for addressing problems

of physiological genetics. We have shown elsewhere (Sorribas & Savageau, 1989*b*) that the use of general constraint relationships provides nothing that cannot be obtained from the solution of the fundamental equations describing the system. On the contrary, the use of constraint relationships actually limits the information that can be obtained.

7. Discussion

It is unfortunate that the relatedness of the various approaches to understanding integrated biochemical systems has not been more widely recognized. The mistaken notion that these approaches are totally unrelated has given rise to considerable misunderstanding. However, most of the confusion can be avoided simply by analyzing the same system with each of the alternatives and making specific comparisons based on objective criteria.

There are several lines of evidence involving such comparisons which demonstrate that BST includes the Kacser-Burns approach as a special case (Savageau *et al.*, 1987*a,b*; Voit & Savageau, 1987; Sorribas & Savageau, 1989*a,b*). BST remains valid under conditions where the summation theorem fails, and for applications that fall within the domain of the Kacser-Burns approach, the two approaches give identical results. We know of no evidence to the contrary. Nevertheless, Kacser & Burns (1979) considered their approach to have superseded BST. No reasons were given, but apparently they thought the summation and connectivity relationship provided fundamentally new information and failed to see that the same information was already available within BST as part of the explicit steady state solution (Savageau, 1971*a*). From the perspective of BST, the summation and connectivity relationships are seen to be a manifestation of the orthogonality properties of the underlying formalism. From the Kacser-Burns perspective, this has been difficult to see, perhaps because the underlying formalism is not made explicit. In any case, a number of recent papers (e.g. Giersch, 1988; Reder, 1988; Sorribas & Savageau, 1989*b*: Cascante *et al.*, 1989*a,b*) now make this manifestation clear when the underlying formalism is implicit rather than explicit.

Thus, the two approaches have much in common throughout their overlapping domain. There are of course differences. Some of these are relatively trivial, but nonetheless, they may obscure the more fundamental similarities if they are not understood. For instance, there are differences in notation and in the convention for numbering reactions of the system. While one might claim certain advantages for one or the other alternative, it also could be argued that these are largely a matter of taste. The differences cannot be considered fundamental, because the same results are obtained in each case and because one can readily translate from one representation to the other. In contrast, other differences have more significant consequences that must be examined objectively and not simply dismissed because the approaches are assumed to be unrelated. For instance, there are differences between implicit and explicit representation of the underlying formalism, differences in strategy for aggregating flux, and differences regarding the independence of individual reactions. With respect to these more important differences, BST is found to have the advantages (Sorribas & Savageau, 1989*a,b,c*).

From our examination of these alternative approaches we conclude that explanations of dominance, and many other phenomena in physiological genetics, cannot be based on the original summation theorem, nor can they be based upon the more general constraint relationships presented in this paper. The general constraint relationships are seen to yield only a subset of the information available directly from the explicit solution. Hence, we do not advocate using the complete set of constraint relationships published in this paper as the foundation for development of physiological genetics. Instead, we recommend that questions of physiological genetics be explored with a stronger theory such as BST, which has predictive power and proven success in other areas of biochemistry and genetics.

This work was supported in part by Grant 135-PIS from the Presidential Initiatives Fund of The University of Michigan, U.S. Public Health Service Grant GM-30054 from the National Institutes of Health, and Grant EET-8712756 from the National Science Foundation.

REFERENCES

BELLMAN, R. (1960). *Introduction to Matrix Analysis.* pp. 90-91. N.Y.: McGraw-Hill.

BODE, H. W. (1945). *Network Analysis and Feedback Amplifier Design.* Princeton, N.J.: Van Nostrand.

BURTON, R. S. & PLACE, A. R. (1986). Evolution of selective neutrality: further considerations. *Genetics* **114,** 1033-1036.

CASCANTE, M., FRANCO, R. & CANELA, E. I. (1989*a*). Use of implicit methods from general sensitivity theory to develop a systematic approach to metabolic control 1. Unbranched chains. *Math. Biosci.* **94,** 271-288.

CASCANTE, M., FRANCO, R. & CANELA, E. I. (1989*b*). Use of implicit methods from general sensitivity theory to develop a systematic approach to metabolic control 2. Complex systems. *Math. Biosci.* **94,** 289-309.

CORNISH-BOWDEN, A. (1987). Dominance is not inevitable. *J. theor. Biol.* **125,** 333-338.

CRABTREE, B. & NEWSHOLME, E. A. (1985). A quantitative approach to metabolic control. *Curr. Top. Cell. Reg.* **25,** 21-76.

CRABTREE, B. & NEWSHOLME, E. A. (1987). The derivation and interpretation of control coefficients. *Biochem. J.* **247,** 113-120.

CROW, J. F. (1987). Sewall Wright and physiological genetics. *Genetics* **115,** 1-2.

CRUZ, J. B. (ed.) (1973). *System Sensitivity Analysis.* Stroudsburg, Pa.: Dowden, Hutchinson and Ross.

FELL, D. A. & SAURO, H. M. (1985). Metabolic control and its analysis. *Eur. J. Biochem.* **148,** 555-561.

FELL, D. A. & SAURO, H. M. (1986). Non-equilibrium/equilibrium reactions: which controls? *Biochem. Soc. Trans.* **14,** 624-625.

GIERSCH, C. (1988). Control analysis of biochemical pathways: a novel procedure for calculating control coefficients and an additional theorem for branched pathways. *J. theor. Biol.* **134,** 451-462.

HARTL, D. L., DEAN, A. M. & DYKHUIZEN, D. E. (1986). The molecular biology of natural selection: reply to Burton and Place. *Genetics* **114,** 1037-1039.

HARTL, D. L., DYKHUIZEN, D. E. & DEAN, A. M. (1985). Limits of adaptation: the evolution of selective neutrality. *Genetics* **111,** 655-674.

HEINRICH, R. & RAPOPORT, T. (1974). A linear steady-state treatment of enzymatic chains. *Eur. J. Biochem.* **42,** 89-95.

HESS, B. & MARKAS, M. (1987). Order and chaos in biochemistry. *Trends Biochem. Sci.* **12,** 45-48.

HOFMEYR, J.-H. S., KACSER, H. & VAN DER MERWE, K. J. (1986). Metabolic control analysis of moiety-conserved cycles. *Eur. J. Biochem.* **155,** 631-641.

IRVINE, D. H. & SAVAGEAU, M. A. (1985*a*). Network regulation of the immune response: alternative control points for suppressor modulation of effector lymphocytes. *J. Immunol.* **134,** 2100-2116.

IRVINE, D. H. & SAVAGEAU, M. A. (1985*b*). Network regulation of the immune response: modulation of suppressor lymphocytes by alternative signals including contrasuppression. *J. Immunol.* **134,** 2117-2130.

IRVINE, D. H. & SAVAGEAU, M. A. Efficient solution of nonlinear ordinary differential equations expressed in S-system canonical form, *SIAM J. Num. Anal.* (in press).

KACSER, H. & BURNS, J. A. (1973). The control of flux. *Symp. Soc. Exp. Biol.* **27,** 65–104.
KACSER, H. & BURNS, J. A. (1979). Molecular democracy: who shares the controls? *Trans. Biochem. Soc.* **7,** 1149–1160.
KACSER, H. & BURNS, J. A. (1981). The molecular basis of dominance. *Genetics* **97,** 639–666.
KACSER, H. & PORTEUS, J. W. (1987). Control of metabolism: what do we have to measure? *Trends Biochem. Sci.* **12,** 5–14.
OKAMOTO, M. & SAVAGEAU, M. A. (1984). Integrated function of a kinetic proofreading mechanism: steady-state analysis testing internal consistency of data obtained *in vivo* and *in vitro* and predicting parameter values. *Biochemistry* **23,** 1701–1709.
REDER, C. (1988). Metabolic control theory: a structural approach. *J. theor. Biol.* **135,** 175–201.
SAVAGEAU, M. A. (1969*a*). Biochemical systems analysis I. Some mathematical properties of the rate law for the component enzymatic reactions. *J. theor. Biol.* **25,** 365–369.
SAVAGEAU, M. A. (1969*b*). Biochemical systems analysis II. The steady state solutions for an *n*-pool system using a power-law approximation. *J. theor. Biol.* **25,** 370–379.
SAVAGEAU, M. A. (1971*a*). Concepts relating the behavior of biochemical systems to their underlying molecular properties. *Arch. Biochem. Biophys.* **145,** 612–621.
SAVAGEAU, M. A. (1971*b*). Parameter sensitivity as a criterion for evaluating and comparing the performance of biochemical systems. *Nature, Lond.* **229,** 542–544.
SAVAGEAU, M. A. (1972). The behavior of intact biochemical control systems. *Curr. Top. Cell. Reg.* **6,** 63–130.
SAVAGEAU, M. A. (1976). *Biochemical Systems Analysis: A Study of Function and Design in Molecular Biology.* Reading, Mass.: Addison Wesley.
SAVAGEAU, M. A. (1979*a*). Autogenous and classical regulation of gene expression: a general theory and experimental evidence. In: *Biological Regulation and Development.* Vol. 1. (Goldberger, R. F., ed.) pp. 57–108, N.Y.: Plenum Press.
SAVAGEAU, M. A. (1979*b*). Growth of complex systems can be related to the properties of their underlying determinants. *Proc. natn. Acad. Sci. U.S.A.* **76,** 5413–5417.
SAVAGEAU, M. A. (1980). A system theory of gene regulation. In: *Proceedings of the 24th Annual North American Meeting of the Society for General Systems Research with the American Association for the Advancement of Science.* (Banathy, B. H., ed.) pp 125–133. Louisville, KY: The Society for General Systems Research.
SAVAGEAU, M. A. (1985). A Theory of alternative designs for biochemical control systems. *Biochim. Biomed. Acta* **44,** 875–880.
SAVAGEAU, M. A. (1987). Control of metabolism: where is the theory? *Trends Biochem. Sci.* **12,** 219–220.
SAVAGEAU, M. A. & JACKNOW, G. (1979). Feedforward inhibition in biosynthetic pathways: inhibition of the aminoacyl-tRNA synthetase by intermediates of the pathway. *J. theor. Biol.* **77,** 405–425.
SAVAGEAU, M. A. & VOIT, E. O. (1987). Recasting nonlinear differential equations as S-systems: a canonical nonlinear form. *Math. Biosci.* **87,** 83–115.
SAVAGEAU, M. A., VOIT, E. O. & IRVINE, D. H. (1987*a*). Biochemical systems theory and metabolic control theory 1. Fundamental similarities and differences. *Math. Biosci.* **86,** 127–145.
SAVAGEAU, M. A., VOIT, E. O. & IRVINE, D. H. (1987*b*). Biochemical systems theory and metabolic control theory 2. The role of summation and connectivity relationships. *Math. Biosci.* **86,** 147–169.
SORRIBAS, A. (1987). Discovering another view of control analysis. *Trends Biochem. Sci.* **12,** 221–222.
SORRIBAS, A. & SAVAGEAU, M. A. (1989*a*). A comparison of variant theories of intact biochemical systems 1: Enzyme-enzyme interactions and biochemical systems theory. *Math. Biosci.* **94,** 161–193.
SORRIBAS, A. & SAVAGEAU, M. A. (1989*b*). A comparison of variant theories of intact biochemical systems 2: Flux oriented and metabolic control theories. *Math. Biosci.* **94,** 195–238.
SORRIBAS, A. & SAVAGEAU, M. A. (1989*c*). Strategies for representing metabolic pathways within biochemical systems theory: Reversible pathways. *Math. Biosci.* **94,** 239–269.
STONER, C. D. (1984). Steady-state kinetics of the overall oxidative phosphorylation reaction in heart mitochondria. Determination of the coupling relationships between the respiratory reactions and miscellaneous observations concerning rate-limiting steps. *J. Bioenerg. Biomembr.* **16,** 115–141.
VOIT, E. O. (1987). Control in perspective. *Trends Biochem. Sci.* **12,** 221.
VOIT, E. O., IRVINE, D. H. & SAVAGEAU, M. A. (1989). *The User's Guide to ESSYNS.* Charleston: Medical University of South Carolina Press.
VOIT, E. O. & SAVAGEAU, M. A. (1982). Power-law approach to modeling biological systems III. Methods of analysis. *J. Ferment. Technol.* **60,** 233–241.
VOIT, E. O. & SAVAGEAU, M. A. (1987). Accuracy of alternative representations for integrated biochemical systems. *Biochemistry* **26,** 6869–6880.
WALEY, S. G. (1964). A note on the kinetics of multi-enzyme systems. *Biochem. J.* **91,** 514–517.

WATT, W. B. (1985). Bioenergetics and evolutionary genetics: opportunities for new synthesis. *Am. Nat.* **125,** 118-143.

WELCH, G. R. & KELETI, T. (1987). Is cell metabolism controlled by a 'molecular democracy' or by a 'supramolecular socialism'? *Trends Biochem. Sci.* **12,** 216-217.

WESTERHOFF, H. V. & CHEN, Y.-D. (1984). How do enzyme activities control metabolic concentrations? *Eur. J. Biochem.* **142,** 425-430.