

Biochemical Systems Theory and Metabolic Control Theory: 2. The Role of Summation and Connectivity Relationships

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ABSTRACT

Perhaps the major obstacle to recognizing the relatedness of Biochemical Systems Theory (BST) and a subsequently developed approach some have called Metabolic Control Theory (MCT) is the summation and connectivity relationships. These are the most visible and central features of the MCT approach to the understanding of intact biochemical systems, whereas in the BST approach they appear to be invisible and peripheral. Generalized versions of these relationships are shown to be inherent to BST, and it is shown how their role differs from that within MCT. The significance of summation and connectivity relationships is shown to be historical and secondary in the sense that one can understand fully the integrated behavior of complex biochemical systems in steady state with BST and never explicitly invoke these relationships. It also is shown that the summation and connectivity relationships in MCT have inherent limitations that make them inadequate as the basis for a general theory of biochemical systems. The results in this paper, together with those in the previous paper, clearly demonstrate that MCT is a special case of BST.

1. INTRODUCTION

Largely as a result of the spectacular advances in molecular biology during the past several decades, a complementary integrative approach to complex biochemical systems has become not only possible but absolutely necessary if we are to understand fully the integrated behavior of such systems. In the late 1960s an integrative approach [13–17] was introduced that represented the first theory to differ significantly from the two well-established approaches based on the Linear Formalism and the Michaelis-Menten Formalism. This approach is called Biochemical Systems Theory (BST); for reviews see Savageau [18, 21–23]. In the mid 1970s another approach was developed [8, 5], which some have referred to as Metabolic

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Control Theory (MCT). For a recent review see [9]. Although MCT appears to cover the same phenomena and to yield the same results as BST, the developers of MCT have treated it as entirely different from BST, and in this regard the field has become confused with seemingly different approaches [28], which in fact are not fundamentally different and which have led to rediscoveries and repetition [31]. This unfortunate situation has developed at least in part because, until very recently, there have been no meaningful cross references between these approaches and no detailed comparisons have been published. Clarification of their relatedness is long overdue.

In the preceding paper [27] we have identified two issues that are central to such clarification: (1) the fundamental character of the formalism that underlies these two approaches, and (2) the role of “summation” and “connectivity” relationships in BST and MCT. The first issue was treated at length in the preceding paper. It was demonstrated that the same Power-Law Formalism underlies both BST and MCT, and that the variant of the Formalism used for BST makes it a more systematic and general theory than MCT.

In this paper we will show that generalized summation and connectivity relationships are inherent to BST. In fact, they will be shown to be mathematically identical to the familiar orthogonality relationships of linear systems. These generalized relationships reduce to the special cases of summation and connectivity relationships found by Kacser and Burns [8] and Heinrich and Rapoport [5] when the restrictions of MCT are taken into account. The role of summation and connectivity relationships within BST and MCT also will be discussed, and further similarities and differences between BST and MCT will be presented.

2. STEADY-STATE SOLUTIONS

In BST the fundamental equations that describe biochemical systems are written explicitly in terms of the underlying Power-Law Formalism [14]:

$$dX_i/dt = \alpha_i \prod_{j=1}^{n+m} X_j^{g_{ij}} - \beta_i \prod_{j=1}^{n+m} X_j^{h_{ij}}, \quad i = 1, \dots, n. \quad (1)$$

The X_i are variables (n are “internal” or dependent and m are “external” or independent); they typically refer to concentrations, but they also may refer to other physical or chemical quantities. The parameters α_i and β_i are rate constants for the net increase (synthesis, import, concentration, etc.) and net decrease (degradation, export, dilution, etc.) of X_i , respectively. The parameters g_{ij} and h_{ij} are kinetic orders that also characterize these net processes.

In steady state, the time derivatives are equal to zero, and Equation (1) can be written as

$$[A]y = b], \quad (2)$$

where

$$\begin{aligned} y_i &= \log X_i, \\ b_i &= \log(\beta_i/\alpha_i), \\ a_{ij} &= g_{ij} - h_{ij}. \end{aligned}$$

Equation (2) has a steady-state solution provided the system determinant is nonzero [14].

The explicit steady-state solution in symbolic form gives the dependent variables and fluxes within an arbitrary system expressed directly in terms of the external or independent variables and molecular parameters associated with the individual enzymes and processes of the system [14]:

$$y]_{\text{in}} = [L]y]_{\text{ex}} + [M]b \quad (3)$$

and

$$\log V] = \log \alpha] + [G]y]. \quad (4)$$

The subscripts “in” and “ex” refer to vectors of internal and external variables, $[L]$ and $[M]$ are matrices whose elements are functions only of the kinetic orders g_{ij} and h_{ij} , V_i is the net flux through the X_i pool, α_i is the corresponding rate constant, and $[G]$ is the matrix of kinetic orders g_{ij} . See Savageau et al. [27] for additional discussion.

Although this solution provides a direct answer to the question of how any dependent variable or flux is determined by the independent variables and molecular parameters, it is convenient for relating elemental components and system behavior also to define particular systemic concepts.

3. SYSTEMIC DESCRIPTIONS

From the explicit symbolic solution in Equations (3) and (4) one can calculate systemic properties directly and can exhibit their relation to the parameters of the underlying molecular mechanisms [16, 17].

3.1. LOGARITHMIC-GAIN FACTORS

The matrix $[L]$ in Equation (3) is an $m \times n$ array of elements that are functions only of the kinetic orders. These elements, defined as logarithmic-gain factors [17, 18] according to well-established precedents [2, 11, 29], are systemic properties that relate the change in the logarithm of an independent variable (X_j) to the resulting change in the logarithm of a dependent variable (X_i):

$$L_{ij} = \frac{\partial \log X_i}{\partial \log X_j} = \frac{\partial y_i}{\partial y_j}. \quad (5)$$

Inspection of these quantities in Equation (3) yields fundamental relationships between elemental components and system behavior: the percentage change in an external variable (X_j) is transmitted throughout the system, and the resultant effect on any given internal variable (X_i) is the original percentage multiplied by the systemic quantity L_{ij} . Hence, the solution in Equation (3) exhibits the relationship between this systemic quantity and the underlying molecular properties, the kinetic orders. For example, see Equation (37). Numerous examples of the use of such relationships also are given elsewhere (e.g., see [18, 21]).

3.2. SENSITIVITIES — RATE CONSTANTS

The matrix $[M]$ in Equation (3) is an $n \times n$ array of elements that also are functions only of the kinetic orders. These elements, defined as sensitivities of dependent variables with respect to rate constants [16, 18], again according to well-established precedents [2, 29, 3], are systemic properties that relate the change in the logarithm of an α or β rate constant to the change in the logarithm of a dependent variable. For example, from Equation (3),

$$y_i = \sum_{j=n+1}^{n+m} L_{ij} y_j + \sum_{j=1}^n M_{ij} (\log \beta_j - \log \alpha_j), \quad i=1, \dots, n, \quad (6)$$

and

$$S(X_i, \alpha_j) = \frac{\partial \log X_i}{\partial \log \alpha_j} = -\frac{\partial y_i}{\partial b_j}, \quad (7)$$

$$S(X_i, \beta_j) = \frac{\partial \log X_i}{\partial \log \beta_j} = \frac{\partial y_i}{\partial b_j}.$$

Thus,

$$S(X_i, \beta_j) = -S(X_i, \alpha_j) = M_{ij}. \quad (8)$$

Inspection of these quantities in Equation (3) yields another class of relationships between elemental components and system behavior: the percentage change in a rate constant parameter (α_j or β_j) is propagated throughout the system, and the resultant effect on any given dependent variable internal to the system (X_i) is the original percentage multiplied by the systemic property M_{ij} . The solution in Equation (3) exhibits the relationship between this systemic property and the underlying molecular properties, the kinetic orders. For example, see Equation (37).

3.3. SENSITIVITIES—KINETIC ORDERS

The effect of variation in kinetic orders can be calculated directly from the steady-state solution in BST. The sensitivity of a dependent variable to a change in kinetic order is defined as

$$S(y_i, g) = \frac{\partial y_i}{\partial g} \frac{g}{y_i}, \quad (9)$$

where g is any given parameter g_{ij} (or h_{ij}) [17, 18]. With this definition expressions comparable to those produced with variation in rate constants can be obtained. In general,

$$\begin{aligned} S(y_i, g) = \frac{\partial y_i}{\partial g} \frac{g}{y_i} &= \frac{\sum_{j=n+1}^{n+m} \frac{\partial L_{ij}}{\partial g} \frac{g}{L_{ij}} L_{ij} y_j + \sum_{j=1}^n \frac{\partial M_{ij}}{\partial g} \frac{g}{M_{ij}} M_{ij} b_j}{\sum_{j=n+1}^{n+m} L_{ij} y_j + \sum_{j=1}^n M_{ij} b_j} \\ &= \frac{\sum_{j=n+1}^{n+m} S(L_{ij}, g) L_{ij} y_j + \sum_{j=1}^n S(M_{ij}, g) M_{ij} b_j}{\sum_{j=n+1}^{n+m} L_{ij} y_j + \sum_{j=1}^n M_{ij} b_j}. \end{aligned} \quad (10)$$

This can be viewed as a weighted average of the sensitivities of the individual coefficients L_{ij} and M_{ij} , which can be seen more clearly when the variables X_i are normalized with respect to their steady-state values. The vector b is then zero, $y]_{in} = [L]y]_{ex}$, and Equation (10) reduces to

$$S(y_i, g) = \frac{\sum_{j=n+1}^{n+m} S(L_{ij}, g) L_{ij} y_j}{\sum_{j=n+1}^{n+m} L_{ij} y_j}. \quad (11)$$

Furthermore, in cases for which there is a single independent variable, or for which all $S(L_{ij}, g)$ are identical,

$$S(y_i, g) = S(L_{ij}, g). \quad (12)$$

These sensitivities have been used in numerous applications of BST (e.g., see [18, 21]). Change in a kinetic order is propagated throughout the system, and the resultant effects are observed in variables internal to the system. The relationship between elemental components and system behavior is made evident by direct calculation from Equation (3).

Any parameter change in a system is ultimately manifested by changes in the two fundamental types of parameters in the Power-Law Formalism—rate

constants and kinetic orders. Although we can determine mathematically the consequences of a change in any of these individual parameters, physical or genetic alteration of a system generally affects several parameters simultaneously [18; 21, Chapter 9; 25; 7]. The net change in any systemic property must therefore be determined by addition of the contributions from each of the affected parameters. We shall see examples in Section 5.

In MCT there is no explicit analytical solution corresponding to Equation (3) and no theorem, corresponding to Equations (13) or (14) in Savageau et al. [27], for the existence of such a steady state. Also the systemic properties corresponding to sensitivities with respect to kinetic orders, which are the most numerous in a system, have not been defined or utilized in MCT. The influence of independent variables on the behavior of the system has been defined in terms of “response coefficients” [8], which are the flux analogs of the logarithmic-gain factors (see Section 3.1) first defined by Savageau [17, 18]. However, the use of logarithmic gains (response coefficients) has not been developed systematically as part of MCT. The influences, termed “concentration control coefficients” (rate-constant sensitivities), of “enzyme levels” (rate constants) on the dependent variables of the system recently have been related directly to the “elasticities” (kinetic orders) in MCT (see [33]). These relationships, however, are not derived directly from an explicit steady-state solution corresponding to Equation (3); rather, these relationships have been derived by use of another set of relationships between sensitivities (control coefficients) and kinetic orders (elasticities)—the summation and connectivity relationships—that are the primitive equations in MCT.

Specific summation and connectivity relationships were found by Kacser and Burns [8] and Heinrich and Rapoport [5]. These relationships were confirmed in BST [21, Chapter 9]. Westerhoff and Chen [33] have found additional relationships of a similar type in MCT. The relationships in BST are generalized summation and connectivity relationships that differ from those in MCT, as will be seen below.

4. ORTHOGONALITY PROPERTIES

Because of the linear structure of the system’s steady-state equations (log form) in BST, the effects of change in the rate constants (or b parameters) can be considered independently of those in the independent y variables [Equation (3)]. For simplicity, and without loss of generality, one may examine the effects of changes in rate constants (b parameters) in an autonomous system, i.e. in a system with no independent variables. The steady-state solution for such a system then can be written

$$y] = [M]b], \quad (13)$$

where $[M] = [A]^{-1}$, and, as previously indicated,

$$M_{ij} = S(X_i, \beta_j) = -S(X_i, \alpha_j). \quad (14)$$

Furthermore, since

$$[M][A] = [I] \quad (\text{identity matrix}), \quad (15)$$

Equation (15) may be thought of as a set of constraints given by

$$\sum_{j=1}^n M_{ij} a_{jk} = \delta_{ik}, \quad (16)$$

where δ_{ik} is the Kronecker delta symbol equal to 1 for $i = k$ and 0 for $i \neq k$. These are the familiar *orthogonality properties* of linear systems (e.g., see [1]).

The generalized summation and connectivity relationships are mathematically identical to these two properties [Equation (14) and Equation (16)].

4.1. SUMMATION — CONCENTRATIONS

Summation and use of Equation (14) yields

$$\sum_{j=1}^n [S(X_k, \alpha_j) + S(X_k, \beta_j)] = \sum_{j=1}^n [S(X_k, \alpha_j) - S(X_k, \alpha_j)] = 0. \quad (17)$$

In words, the percentage change in some dependent variable X_k resulting from a one-percent change in the rate constant for a given net reaction or process, summed over all such reactions or processes in the system, must be zero.

This is a general constraint. When there are no branches in the system, and when the rate laws are independent of each other and linear functions of enzyme levels, then this generalized summation relationship reduces to the special case published by Heinrich and Rapoport [5]:¹

$$\sum_{j=1}^{2n} C_{E_j}^{X_k} = 0, \quad (18)$$

where

$$C_{E_j}^{X_k} = S(X_k, \alpha_j) \text{ [or } S(X_k, \beta_j)].$$

¹If there are branches in the flow of material and precursor-product constraints, but the rate laws still are independent and linear in enzyme concentration, then the corresponding relationships in BST and MCT are still equally valid. However, they involve different interpretations, as we shall see in Section 5. If the rate laws are not independent or not linear in enzyme concentration, then the relationships in MCT are no longer valid.

Note that in this case biosynthetic and degradative terms are not distinguished notationally, and thus E is used as an analogy for both α and β .

4.2. CONNECTIVITY—CONCENTRATIONS

Substitution of the relationships in Equation (14) into Equation (16) yields

$$\sum_{j=1}^n S(X_i, \beta_j) a_{jk} = \delta_{ik},$$

or

$$\sum_{j=1}^n [S(X_i, \beta_j) g_{jk} - S(X_i, \beta_j) h_{jk}] = \delta_{ik},$$

since $a_{jk} = g_{jk} - h_{jk}$. Again using the relationships in Equation (14), one finds

$$\sum_{j=1}^n [S(X_i, \alpha_j) g_{jk} + S(X_i, \beta_j) h_{jk}] = -\delta_{ik}. \quad (19)$$

In words, when the percentage change in some dependent variable X_i resulting from a one-percent change in the rate constant for a given net reaction or process is multiplied by the kinetic order of that net reaction or process with respect to another dependent variable X_k and these products are summed over all net reactions or processes in the system, the result is equal to zero if $i \neq k$ or minus one if $i = k$.

These results are true in general. When there are no branches in the system, and when the rate laws are independent of each other and linear functions of enzyme levels, then this generalized connectivity relationship reduces to the special case published by Westerhoff and Chen [33] (see footnote 1):

$$\sum_{j=1}^{2n} C_{E_j}^{X_i} \epsilon_{X_k}^j = -\delta_{ik}. \quad (20)$$

Note that because biosynthetic and degradative reactions are not distinguished notationally, ϵ is used for both g and h .

4.3. *SUMMATION — FLUXES*

Historically, specific relationships of this type were among the first of the summation and connectivity relationships to be found [8, 5]. The verification of these relationships in BST [21, Chapter 9] was accomplished by first establishing the concentration relationships in the previous two sections. The procedure is as follows.

An arbitrary flux, V_k , through the pool X_k is given by

$$V_k = \alpha_k \prod_{j=1}^n X_j^{g_{kj}}. \quad (21)$$

By definition

$$S(V_k, \alpha_i) = \frac{\partial \log V_k}{\partial \log \alpha_i} = \delta_{ik} + \sum_{j=1}^n g_{kj} S(X_j, \alpha_i),$$

$$S(V_k, \beta_i) = \frac{\partial \log V_k}{\partial \log \beta_i} = \sum_{j=1}^n g_{kj} S(X_j, \beta_i). \quad (22)$$

When these terms are summed,

$$\sum_{i=1}^n [S(V_k, \alpha_i) + S(V_k, \beta_i)]$$

$$= 1 + \sum_{i=1}^n \sum_{j=1}^n g_{kj} [S(X_j, \alpha_i) + S(X_j, \beta_i)] = 1 \quad (23)$$

The percentage change in the given flux resulting from a one-percent change in the rate constant of a net reaction or process, summed over all such reactions or processes, must be unity.

This is a general result, which, when there are no branches in the system, and when the rate laws are independent of each other and linear functions of enzyme levels, reduces to the special case first published by Kacser and Burns [8] and Heinrich and Rapoport [5] (see footnote 1):

$$\sum_{i=1}^{2n} C_{E_i}^{J_k} = 1. \quad (24)$$

4.4. *CONNECTIVITY — FLUXES*

Specific relationships of this type also were first published by Kacser and Burns [8], and again the verification in BST proceeded from the relationships

in Sections 4.1 and 4.2. Thus,

$$\begin{aligned}
 & \sum_{i=1}^n [S(V_k, \alpha_i) g_{im} + S(V_k, \beta_i) h_{im}] \\
 &= \sum_{i=1}^n \left\{ \left[\delta_{ik} + \sum_{j=1}^n g_{kj} S(X_j, \alpha_i) \right] g_{im} + \left[\sum_{j=1}^n g_{kj} S(X_j, \beta_i) \right] h_{im} \right\} \\
 &= g_{km} - \sum_{i=1}^n \sum_{j=1}^n g_{kj} S(X_j, \beta_i) a_{im} \\
 &= g_{km} - \sum_{j=1}^n g_{kj} \left[\sum_{i=1}^n S(X_j, \beta_i) a_{im} \right] \\
 &= g_{km} - \sum_{j=1}^n g_{kj} [\delta_{jm}] = 0. \tag{25}
 \end{aligned}$$

When the percentage change in an arbitrary flux V_k through the pool X_k resulting from a one-percent change in the rate constant for a given net reaction or process is multiplied by the kinetic order of that reaction or process with respect to an arbitrary dependent variable X_m and summed over all such reactions or processes, the result must be zero.

Again, this is a general relationship, which under appropriate circumstances reduces to the special case described by Kacser and Burns [8] (see footnote 1):

$$\sum_{i=1}^{2n} C_{E_i}^j \epsilon_{X_m}^i = 0. \tag{26}$$

The results in this section show that constraint relations similar to, but more general than, the specific summation and connectivity relationships first described in MCT are inherent to the structure of the Power-Law Formalism that underlies both BST and MCT. These relationships provide another interesting perspective on the Power-Law Formalism, but they have not added fundamentally to or extended the range of application for BST. The complete solution to the problem of relating systemic behavior to underlying molecular determinants in steady state was provided by BST without the explicit use of the relationships in this section [17].²

²From our demonstration that summation and connectivity relationships are none other than the familiar orthogonality relationships of linear systems, it is clear that any attempt to “prove” these relationships by first solving a set of linear equations for the sensitivities [control coefficients] (e.g., see Kacser and Porteous [9] and references in their “Reply” following Savageau [24]) is a circular argument. The solution of such equations requires orthogonality (or summation and connectivity) relationships in the first place.

5. BRANCHED BIOSYNTHETIC PATHWAY—AN EXAMPLE

Although unbranched biosynthetic pathways appear to be governed by a single pattern of regulation, which by several criteria for functional effectiveness represents an optimal pattern [18, 19], branched biosynthetic pathways exhibit a diversity of patterns of regulation that are poorly understood. A predominant pattern in enteric bacteria, the nested pattern of regulation, has been analyzed in some detail [18; 21, Chapter 12]. The results of this analysis led to the prediction of multifunctional enzyme complexes among key enzymes and to a clear rationale in terms of regulatory purpose. Experimental evidence in support of these predictions has been discussed elsewhere [21, Chapter 12]. These results also led to the prediction of a new regulatory interaction in a system that only partially fulfilled the requirements for the normal nested pattern of regulation [10].

5.1. REPRESENTATION IN BST

Figure 1 represents a simplified model of such a branched biosynthetic system. The distinguishing features of this model are the existence of precursor-product relationships, branches in the flow of material, and complex regulatory interactions. The equations that describe this system in BST are

$$\begin{aligned} dX_1/dt &= \alpha_1 X_0^{g_{10}} X_2^{g_{12}} X_3^{g_{13}} - \beta_1 X_1^{h_{11}} X_2^{h_{12}} X_3^{h_{13}}, \\ dX_2/dt &= \alpha_2 X_1^{g_{21}} X_2^{g_{22}} - \beta_2 X_2^{h_{22}}, \\ dX_3/dt &= \alpha_3 X_1^{g_{31}} X_3^{g_{33}} - \beta_3 X_3^{h_{33}}. \end{aligned} \quad (27)$$

The rate laws for the two processes degrading X_1 have been combined into a net rate law, and then the system has been described in the Power-Law Formalism. As noted earlier [27], this description provides the natural

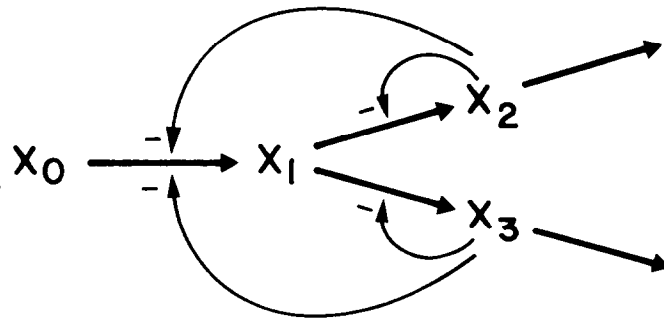


FIG. 1. Branched biosynthetic pathway. X_0 is an externally determined, independent concentration variable. The arrows from the dependent concentration variables X_2 and X_3 to the center of other arrows represent inhibitory interactions.

parameters for expressing the steady-state existence [14; 21, Chapter 7] and local stability [20; 21, Chapter 7] theorems, namely, the differences between the kinetic orders for net synthesis and net degradation ($g_{ij} - h_{ij}$). It also makes transparent the relationships between the sensitivities with respect to the rate constants for synthetic and degradative reactions.

Although there are precursor-product relationships, one cannot equate parameters such as h_{12} and g_{22} or h_{13} and g_{33} , because the net degradation of X_1 is not the same as the net synthesis of X_2 (or X_3). This is one reason why different symbols were originally introduced in BST [14; 18; 21, Chapter 12]. Nevertheless, the precursor-product constraints do lead to obvious relationships among these parameters. At steady-state operating points for this system

$$\beta_1 X_{10}^{h_{11}} X_{20}^{h_{12}} X_{30}^{h_{13}} = \alpha_2 X_{10}^{g_{21}} X_{20}^{g_{22}} + \alpha_3 X_{10}^{g_{31}} X_{30}^{g_{33}}, \quad (28)$$

and by definition of kinetic order [14, 17]

$$h_{11} = \frac{V_2}{V_1} g_{21} + \frac{V_3}{V_1} g_{31}, \quad (29)$$

$$h_{12} = \frac{V_2}{V_1} g_{22}, \quad (30)$$

$$h_{13} = \frac{V_3}{V_1} g_{33}; \quad (31)$$

also

$$\beta_1 = (V_2 + V_3) X_{10}^{-h_{11}} X_{20}^{-h_{12}} X_{30}^{-h_{13}}, \quad (32)$$

where

$$V_2 = \alpha_2 X_{10}^{g_{21}} X_{20}^{g_{22}}, \quad (33)$$

$$V_3 = \alpha_3 X_{10}^{g_{31}} X_{30}^{g_{33}}, \quad (34)$$

$$V_1 = V_2 + V_3. \quad (35)$$

In words, the kinetic order with respect to a given X_i in the net rate law is simply the sum of the corresponding kinetic orders in the individual rate laws, weighted according to the contributions of the individual fluxes to the net flux.

5.2. EXPLICIT STEADY-STATE SOLUTION IN BST

As in Section 2, the steady-state equations describing this system in logarithmic form can be written in a straightforward manner. If the determinant of the system matrix is nonzero, i.e.,

$$|A| = -(h_{11} a_{22} a_{33} + g_{31} a_{22} a_{13} + g_{21} a_{33} a_{12}) \neq 0, \quad (36)$$

then a steady-state solution exists and can be written explicitly [cf. Equation (3)]:

$$\begin{bmatrix} y_1 \\ y_2 \\ y_3 \end{bmatrix} = \frac{1}{|A|} \begin{bmatrix} -g_{10} a_{22} a_{33} \\ g_{10} g_{21} a_{33} \\ g_{10} g_{31} a_{22} \end{bmatrix} y_0 + \frac{1}{|A|} \begin{bmatrix} a_{22} a_{33} & -a_{12} a_{33} & -a_{13} a_{22} \\ -g_{21} a_{33} & -(h_{11} a_{33} + g_{31} a_{13}) & g_{21} a_{13} \\ -g_{31} a_{22} & g_{31} a_{12} & -(h_{11} a_{22} + g_{21} a_{12}) \end{bmatrix} \begin{bmatrix} b_1 \\ b_2 \\ b_3 \end{bmatrix}. \quad (37)$$

Thus, the parameter values and the value of the independent concentration variable X_0 determine the nonzero steady-state values of the dependent concentration variables X_1 , X_2 , and X_3 . From these equations one can calculate directly (or see by inspection) the change in dependent concentrations that would result from a change in independent concentration ($L_{i,0}$), a change in kinetic order [$S(y_i, h_{ik})$], or a change in rate constant [$S(X_i, \beta_j)$].

As in Section 3, the systemic properties (logarithmic gains and sensitivities) can be calculated directly and expressed in terms of the underlying molecular parameters (α 's, β 's, g 's, and h 's). One also can invert these relationships and express the underlying molecular parameters in terms of the systemic properties, although in some cases this calculation requires additional information about changes in fluxes. In this example

$$\begin{aligned} h_{11} &= \frac{M_{23} M_{32} - M_{22} M_{33}}{|M|}, \\ g_{21} &= \frac{M_{23} M_{31} - M_{21} M_{33}}{|M|}, \\ g_{31} &= \frac{M_{21} M_{32} - M_{22} M_{31}}{|M|}, \\ g_{10} &= -\frac{L_{30}}{M_{31}}, \\ a_{12} &= \frac{M_{13} M_{32} - M_{12} M_{33}}{|M|}, \\ a_{22} &= \frac{M_{11} M_{33} - M_{13} M_{31}}{|M|}, \\ a_{13} &= \frac{M_{12} M_{23} - M_{13} M_{22}}{|M|}, \\ a_{33} &= \frac{M_{11} M_{22} - M_{12} M_{21}}{|M|}, \end{aligned} \quad (38)$$

$$|M| = M_{11}M_{22}M_{33} + M_{12}M_{23}M_{31} + M_{13}M_{32}M_{21} \\
 - M_{11}M_{23}M_{32} - M_{22}M_{13}M_{31} - M_{33}M_{12}M_{21}.$$

The first four kinetic orders in Equation (38) are uniquely related to the systemic properties; the remaining eight are not related uniquely but in pairs ($g_{ij} - h_{ij}$) to the systemic properties. Measurements of changes in steady-state fluxes can be used to obtain the individual molecular parameters. For example, the parameters β_3 and h_{33} in Equation (27) can be obtained from measurements of V_3 and X_3 by linear regression according to the formula

$$\log V_3 = \log \beta_3 + h_{33} \log X_3. \quad (39)$$

The conditions governing local dynamic stability require that

$$|A| = -(h_{11}a_{22}a_{33} + g_{31}a_{22}a_{13} + g_{21}a_{33}a_{12}) < 0 \quad (40)$$

for the system to be stable [18; 20; 21, Chapter 7]. The local dynamic behavior of the system follows from Equation (27) and has been explored elsewhere [21, Chapter 12]. As we shall see in Section 5.6, the condition in Equation (40) also is essential for interpreting the direction of change in variables as the system moves from one steady state to another.

5.3. SUMMATION AND CONNECTIVITY RELATIONSHIPS IN BST

One easily can verify that all the summation and connectivity relations are satisfied. For example, the concentration summation for X_2 is given by

$$S(X_2, \alpha_1) + S(X_2, \beta_1) \\
 + S(X_2, \alpha_2) + S(X_2, \beta_2) + S(X_2, \alpha_3) + S(X_2, \beta_3) = 0$$

or

$$g_{21}a_{33} - g_{21}a_{33} + (h_{11}a_{33} + g_{31}a_{13}) - (h_{11}a_{33} + g_{31}a_{13}) \\
 - g_{21}a_{13} + g_{21}a_{13} = 0 \quad (41)$$

by inspection of Equation (37). Note that the determinant $|A|$ common to each sensitivity has been canceled for simplicity. Because the sensitivities with respect to net synthesis and net degradation are always equal in magnitude but opposite in sign, this is satisfied trivially. However, the precursor-product constraints prevent one from physically altering the parameters α_2 or α_3 without concomitantly changing the parameter β_1 . Thus, the second sensitivity in Equation (41) actually is composed of two components; the sum of one of these and the third sensitivity in Equation (41) will represent the sensitivity of X_2 with respect to the physical change in

α_2 , while the sum of the remaining component and the fifth sensitivity in Equation (41) will represent the sensitivity of X_2 with respect to the physical change in α_3 . Again, these individual contributions are clearly distinguished mathematically even though they cannot be distinguished physically. We can see these relationships explicitly if we express this summation relationship after imposing the precursor-product constraints [Equations (32)–(34)] and making the appropriate calculations:

$$S(X_2, \alpha_1) + S'(X_2, \alpha_2) + S(X_2, \beta_2) + S'(X_2, \alpha_3) + S(X_2, \beta_3) = 0,$$

or

$$\begin{aligned}
 g_{21}a_{33} + \left[(h_{11}a_{33} + g_{31}a_{13}) - \frac{V_2}{V_1}g_{21}a_{33} \right] - (h_{11}a_{33} + g_{31}a_{13}) \\
 - \left[g_{21}a_{13} + \frac{V_3}{V_1}g_{21}a_{33} \right] + g_{21}a_{13} = 0,
 \end{aligned} \tag{42}$$

where again $|A|$ has been canceled for convenience.

Comparison of Equations (41) and (42) shows that

$$S'(X_2, \alpha_2) = S(X_2, \alpha_2) + \frac{V_2}{V_1}S(X_2, \beta_1), \tag{43}$$

$$S'(X_2, \alpha_3) = S(X_2, \alpha_3) + \frac{V_3}{V_1}S(X_2, \beta_1). \tag{44}$$

The terms in the brackets in Equation (42) represent the sensitivity to a physical change in α_2 and α_3 . Within each bracket one can identify the individual contributions from the effects on synthesis and degradation, even though these individual effects cannot be physically separated. The branching in this example implies that the synthesis of each end product is only equivalent to the relevant fraction of the degradation of the branchpoint metabolite X_1 . Thus, the two degradative contributions are weighted according to the fraction of the total flux in each branch.

Other summation and connectivity relations could be examined in the same fashion.

5.4. REPRESENTATION IN MCT

As we have seen, the general solution in BST [17] applies directly to systems with branches. For these systems, MCT reached essentially the same solution when Heinrich and Rapoport [6] provided the additional relationships needed in MCT to deal with branches:

$$\alpha_{X_r} V_r + \sum_{i=1}^n \sum_{k=1}^m \alpha_{X_i} V_i \epsilon_{X_k}^i C_{E_r}^{X_k} = 0 \tag{45}$$

$$JC_{E_r}^J = \sum_{i=1}^n \sum_{k=1}^m \beta_{J_i} V_i \epsilon_{X_k}^i C_{E_r}^{X_k} + \beta_{J_r} V_r. \quad (46)$$

The calculation of a typical sensitivity (control coefficient) in MCT proceeds in a very different fashion from that illustrated above for BST. In MCT the branched system in Figure 1 is described by the following equations (see [27]):

$$\begin{aligned} dX_1/dt &= E_1 X_0^{\epsilon_{X_0}^1} X_2^{\epsilon_{X_2}^1} X_3^{\epsilon_{X_3}^1} - E_4 X_1^{\epsilon_{X_1}^4} X_2^{\epsilon_{X_2}^4} - E_5 X_1^{\epsilon_{X_1}^5} X_3^{\epsilon_{X_3}^5}, \\ dX_2/dt &= E_4 X_1^{\epsilon_{X_1}^4} X_2^{\epsilon_{X_2}^4} - E_2 X_2^{\epsilon_{X_2}^2}, \\ dX_3/dt &= E_5 X_1^{\epsilon_{X_1}^5} X_3^{\epsilon_{X_3}^5} - E_3 X_3^{\epsilon_{X_3}^3}, \end{aligned} \quad (47)$$

the fundamental difference being the application of the Power-Law Formalism before summing the individual rates of degradation for X_1 [cf. Equation (27)]. In this MCT form there is no general or symbolic steady-state solution that can be obtained explicitly. Consequently there are no simple steady-state existence and local stability theorems corresponding to Equations (36) and (40) that can be stated explicitly in terms of kinetic orders and rate constants.

5.5. SUMMATION AND CONNECTIVITY RELATIONSHIPS IN MCT

Relationships between elemental component and system behavior in MCT are obtained by use of the summation and connectivity relationships. In general there are n summation relationships [Equation (18)] and $n \times n$ connectivity relationships [Equation (20)] for n concentration variables, and N summation relationships [Equation (24)] and $n \times N$ connectivity relationships [Equation (26)] for N flux variables. In examples such as this, which involve branches in the flow of material, one also must use the auxiliary relationships developed by Heinrich and Rapoport [6]. In general there are $n \times N$ relating concentration variables [Equation (45)] and $N \times N$ relating flux variables [Equation (46)]. The resulting set of equations is highly redundant, and one must find a linearly independent set (e.g., see [1]) if one is to obtain a unique solution.

As an illustration let us relate the systemic property $C_{E_3}^{X_2}$ to the elasticities and fluxes of the system in steady state. In this case there are 72 equations specified by Equations (18), (20), (24), (26), (45), and (46). We will not go through the details of determining a linearly independent set that will allow

one to solve uniquely for $C_{E_3}^{X_2}$, but simply indicate that the following three equations will suffice.

When $s = 1$ and $r = 3$, Equation (45) implies

$$-(V_4 \epsilon_{X_1}^4 + V_5 \epsilon_{X_1}^5) C_{E_3}^{X_1} + (V_1 \epsilon_{X_2}^1 - V_4 \epsilon_{X_2}^4) C_{E_3}^{X_2} + (V_1 \epsilon_{X_3}^1 - V_5 \epsilon_{X_3}^5) C_{E_3}^{X_3} = 0. \quad (48)$$

Similarly, when $s = 2$ and $r = 3$,

$$V_4 \epsilon_{X_1}^4 C_{E_3}^{X_1} + (V_4 \epsilon_{X_2}^4 - V_2 \epsilon_{X_2}^2) C_{E_3}^{X_2} = 0, \quad (49)$$

and when $s = 3$ and $r = 3$,

$$-V_3 + V_5 \epsilon_{X_1}^5 C_{E_3}^{X_1} + (V_5 \epsilon_{X_3}^5 - V_3 \epsilon_{X_3}^3) C_{E_3}^{X_3} = 0. \quad (50)$$

These represent three linear equations in the three control coefficients, and thus can be solved under appropriate conditions for the coefficient of interest:

$$\begin{aligned} C_{E_3}^{X_2} = & - \left[\epsilon_{X_1}^4 \left(\epsilon_{X_3}^1 - \frac{V_5}{V_1} \epsilon_{X_3}^5 \right) \right] \\ & \times \left[\left(\frac{V_4}{V_1} \epsilon_{X_1}^4 + \frac{V_5}{V_1} \epsilon_{X_1}^5 \right) (\epsilon_{X_2}^4 - \epsilon_{X_2}^2) (\epsilon_{X_3}^5 - \epsilon_{X_3}^3) \right. \\ & + \epsilon_{X_1}^5 (\epsilon_{X_2}^4 - \epsilon_{X_2}^2) \left(\epsilon_{X_3}^1 - \frac{V_5}{V_1} \epsilon_{X_3}^5 \right) \\ & \left. + \epsilon_{X_1}^4 (\epsilon_{X_3}^5 - \epsilon_{X_3}^3) \left(\epsilon_{X_2}^1 - \frac{V_4}{V_1} \epsilon_{X_2}^4 \right) \right]^{-1}. \quad (51) \end{aligned}$$

One would have to go through a similar process, although perhaps involving a different set of equations, for each coefficient of interest.

Fell and Sauro [4] have described a simpler alternative for generating a set of linearly independent equations in MCT; the same results are obtained by either method.

5.6. COMPARISON OF RESULTS FROM MCT AND BST

The results in Section 5.5, although expressed in symbolic form, do not allow one to draw general conclusions, such as the direction of change in X_2 following an increase in E_3 , that are independent of the particular parameter values. This is because the sign of the denominator in Equation (51) is indeterminate.

One can compare this result in MCT with the corresponding result in BST by noting the following relationships between their symbols:

$$\begin{aligned} h_{11} &= \frac{V_4}{V_1} \varepsilon_{X_1}^4 + \frac{V_5}{V_1} \varepsilon_{X_1}^5, \\ h_{12} &= \frac{V_4}{V_1} \varepsilon_{X_2}^4, \\ h_{13} &= \frac{V_5}{V_1} \varepsilon_{X_3}^5. \end{aligned} \quad (52)$$

The other elasticities can be translated directly into the corresponding kinetic orders, and Equation (51) reduces to

$$S(X_2, \beta_3) = - \frac{g_{21} a_{13}}{h_{11} a_{22} a_{33} + g_{31} a_{22} a_{13} + g_{21} a_{33} a_{12}}, \quad (53)$$

which could have been obtained simply by inspection of Equation (37).

Although the alternative expressions in Equations (51) and (53) are mathematically equivalent, the attainment, biological meaning, and interpretation of Equation (53) are more transparent. All sensitivities with respect to changes in enzyme levels [e.g., Equation (53)] can be obtained directly from the general symbolic solutions in Equation (37). Equation (53) illustrates the fundamental importance of the differences between kinetic orders for net synthesis and net degradation, which are made explicit in BST. In this example, the relative magnitudes of g_{13} and h_{13} (in $a_{13} = g_{13} - h_{13}$) determine completely the direction of change in X_2 . An increase in the amount of the enzyme degrading X_3 causes a change in the steady-state value of the other product X_2 . If the system is stable, then the denominator in Equation (53) is positive (regardless of the particular values of the parameters). The parameter g_{21} is positive, so X_2 will either increase or decrease depending upon the sign of the parameter a_{13} . If $a_{13} < 0$ ($g_{13} < h_{13}$), then X_2 will increase; if $a_{13} > 0$ ($g_{13} > h_{13}$), then X_2 will decrease. Without the stability condition [Equation (40)] to show that the denominator is positive, one would have no way to predict the direction of change in X_2 .

This example illustrates the analysis of a general class of biochemical systems and shows that one can make general predictions that are independent of the particular numerical values for the specific parameters. It also shows clearly the advantages of aggregating rate laws for net synthesis and net degradation (see also [32]), and the importance of the stability relationships [18; 20; 21, Chapter 12] for interpreting steady-state results.

6. DISCUSSION

We began the previous paper by noting that the original presentation and subsequent development of MCT have not acknowledged the earlier

results in BST nor shown how these have been augmented in MCT [27]. A possible explanation is that MCT and BST were seen as unrelated because of differences in the level of formal development and in the existence of specific summation and connectivity relationships. This impression persisted because MCT had never been explicitly expressed in terms of the fundamental, underlying mathematical structure and because it had never been systematically compared with the earlier BST.

The results in the previous paper demonstrated that in fact the above differences are rather superficial and that at a fundamental level the logical content of MCT is a subset of that of BST. BST is a more comprehensive theory, has a more systematic structure based explicitly on the underlying formalism, and appears to have a broader range of valid application [32].

Perhaps the major stumbling block to recognizing MCT's relatedness to BST is the summation and connectivity relationships. These are the most visible and central feature of MCT, while in BST they appear to be invisible and peripheral.

In this paper we have elaborated on the generalized summation and connectivity relationships in BST that were used to verify [21, Chapter 9] the specific relationships described by Kacser and Burns [8] and Heinrich and Rapoport [5]. It is demonstrated that these generalized relationships are a natural part of BST. They are equivalent to the orthogonality properties, which are inherent to the steady-state equations in the Power-Law Formalism because of their linear structure, and they are expressed in terms of the fundamental parameters: rate constants and kinetic orders. In contrast, special versions of such relationships were presented in an *ad hoc* fashion and then made the basis of MCT [8, 5, 9]. These specific relationships were expressed in terms of kinetic orders (elasticities) and enzyme levels or molecular activities. The latter are not fundamental parameters in the underlying formalism, and the resulting relationships are valid only for systems in which the rate laws for the individual enzymes are independent of each other and are linear functions of enzyme concentration and molecular activity. As we have seen, BST and MCT do *not* differ in that one has summation and connectivity relationships while the other does not; these types of relationships exist in both approaches. Nevertheless, these relationships play significantly different roles in BST and MCT.

As pointed out in this paper, summation and connectivity relationships have no operational role in BST. They are only corollaries in BST, and need never be made manifest during the development of the theory or for purposes of application. In this sense they play no significant role. One can do everything directly in BST and never explicitly invoke the summation or connectivity relationships. For this reason we have never thought it important to highlight these relationships, except to note that they can be verified readily in BST [21, Chapter 9].

In contrast, summation and connectivity relationships have held a special place in MCT. Two distinct roles can be identified: they serve as “conservation laws,” and they serve as the “primitive equations” for relating systemic and molecular properties, and thus as the port of entry into the theoretical domain it shares with BST.

The original development and use of the summation and connectivity relationships within MCT occurred in the context of sequential chains of simple enzymatic reactions [8, 5], and this gave rise to the notion of sensitivities (control coefficients) as positive fractional quantities that must sum to one for the flux in the system. Sensitivity (“control”) is distributed or shared among all the enzymes of the system. If one enzyme has a sensitivity (control coefficient) of one, then it would be “controlling” and all others would have values of zero. There is a “unit” amount of “control” and it is conserved. If the value goes up in one part of the system, then it must go down elsewhere. In this sense these quantities might be considered analogous to energy, and the summation relationships to the conservation of energy.

One might use such a “conservation law” to test the validity of a model. If the experimentally determined sensitivities (control coefficients) for each of the processes in the model do not add up to one, then the model is lacking some important element. If they do, then the model is complete. However, this role for summation relationships can be criticized on several levels.

First, this role can only be fulfilled within the limited context of sequential chains of simple enzymatic reactions, and then only if one tabulates *experimentally determined* values (e.g. see [30]). In cases where the values are *calculated from the model* (e.g., see [12]) the test is no longer valid, since, as noted earlier, these relationships always will sum to unity—regardless of whether the model is valid or invalid. It is only the discrepancy between experimental measurements and unity (the model result) that is of diagnostic value.

Second, sensitivities (control coefficients) need not have positive fractional values when more complex systems, such as those containing enzyme cascades [17; 21, Chapter 13, 15, 16] or branches in the flow of material [18; 21, Chapter 12], are considered. When sensitivities (control coefficients) can be negative as well as positive and when they can have magnitudes greater than unity, the ability to test for completeness of a model is lost. Even if experimentally determined values sum to unity, one might have omitted several important elements from the model; positive and negative contributions from neglected elements can cancel fortuitously. There can be several enzymes with sensitivities (control coefficients) greater than or equal to unity, and others need not have values equal to zero. All that remains is the individual sensitivities, and the “conservation laws” do not allow one to deduce directly the relationship among their values, as they do for simpler systems.

Third, the summation and connectivity relationships in MCT are expressed in terms of enzyme activities, enzyme levels, or molecular activities [8, 5, 9]; these are not valid except for simple systems in which the rate laws are independent of each other and proportional to enzyme concentration or molecular activity [24, 27]. Thus, in more general cases the idea of "conservation law" is meaningless.

The more fundamental role of the summation and connectivity relationships in MCT is that of "primitive equations" used to obtain the relationship between systemic and molecular properties in biochemical systems. This role also is subject to several levels of criticism. First, these summation and connectivity relationships are not sufficient to obtain the explicit relationships between systemic and molecular parameters, except in the special case of sequential chains of simple enzymatic reactions [8, 5]. One has to introduce auxiliary relationships [6] in the case of branching systems in order to obtain in MCT a solution comparable to that obtained earlier in BST [14, 17]. Second, the summation and connectivity relationships in MCT do not give conditions for the existence or the local stability of a steady state. As the basis for a general theory, these summation and connectivity relationships in MCT are therefore inferior to the underlying equations of BST, which do yield such conditions [14, 20]. Third, and most fundamental, these summation and connectivity relationships are based on rate laws that are assumed to be independent of each other and linearly related to enzyme level and molecular activity [8, 5, 9]. Since biological systems are known to contain reactions that do not fit this simplifying assumption [24, 27], these summation and connectivity relationships are not generally valid. They do have an intuitive appeal if they are accepted uncritically, which may be fostered by claims [9] that they are entirely general. To the best of our knowledge, the conditions identified here, which are required for the validity of the summation and connectivity relationships in MCT, have never been verified in any application.

From these results one can conclude that MCT is not a new theory unrelated to BST. In fact, MCT is but a special case or subset of BST. By attempting to make specific summation and connectivity relationships the basis of a theory (MCT) one is prevented from recognizing the explicit structure of the underlying formalism. Paradoxically, one also is prevented from recognizing the more general expression and interpretation of summation and connectivity relationships themselves. As we have seen, the rate-constant parameters that are essential for the more general expression of these relationships are not present in MCT. The sensitivities with respect to the most numerous parameters in a system—the kinetic orders—also are absent from MCT.

Finally, it should be pointed out that BST, by focusing upon the explicit structure of the underlying formalism, has led to a natural extension of the

theory from approximate descriptions and local ranges of valid application to "exact" descriptions and global ranges of valid application. This extension of the theory is beyond the scope of this paper and is published elsewhere (for a review see Savageau and Voit [26]).

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