J. theor. Biol. (1991) **149,** 521–535

Biochemical Systems Theory: Increasing Predictive Power by using Second-order Derivatives Measurements

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(*Received on* 14 *May* 1990, *Accepted in revised form on* 27 *July* 1990)

Models based on the power-law formalism provide a useful tool for analyzing metabolic systems. Within this methodology, the S-system variant furnishes the best strategy. In this paper we explore an extension of this formalism by considering second-order derivative terms of the Taylor series which the power-law is based upon. Results show that the S-system equations which include second-order Taylor coefficients give better accuracy in predicting the response of the system to a perturbation. Hence, models based on this new approach could provide a useful tool for quantitative purposes if one is able to measure the required derivatives experimentally. In particular we show the utility of this approach when it comes to discriminating between two mechanisms that are equivalent in the S-system a representation based on first-order coefficients. However, the loss of analytical tractability is a serious disadvantage for using this approach as a general tool for studying metabolic systems.

Introduction

Among the several formalisms developed for representing a metabolic process, the power-law formalism (Savageau, 1969*a*, *b*, 1970) has several advantages: (1) it is simple; (2) it is systematic; and (3) it yields a useful representation of the system. Using this formalism, the rate of any process V_i, is written:

$$V_i = \alpha_i \prod_{j=1}^{n+m} X_j^{g_{ij}}. \tag{1}$$

In this equation, X_j ($j = 1, \ldots, n$ for dependent variables; $j = n+1, \ldots, n+m$ for independent variables) are varibles (enzymes, metabolites, effectors, etc) affecting the considered rate. In eqn (1) parameters have a definite meaning, that of kinetic order (g_{ij}) and rate constant (α_i) (see Sorribas & Savageau, 1989*a* and references therein for a discussion of these parameters).

This formalism results from a Taylor series expansion of the actual rate law in a log–log space (Savageau, 1969*b*), after considering the linear terms. Hence, eqn (1) is an approximated representation of the considered process at a specific operating

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0022-5193/91/080521+15 $03.00/0

point defined by $X_{1(0)}, \ldots, X_{n+m(0)}$. Consequently, theory assures us that this equation will accurately represent this process in the vicinity of this point, even though its accuracy will decrease as we move away from it. The range of variation leading to the preservation of accuracy within a definite limit will depend on the specific properties of the considered process and on the operating point (Voit & Savageau, 1987; Sorribas & Savageau, 1989*a*, *c*).

In this sense, it is important to recall that the power-law formalism is not intended to provide an alternative representation for kinetic studies *in vitro*, but a valid mathematical representation of the integrated system's behavior. Of course, if we are interested in accurately representing the functional dependence of V_i on each X_j for the full range of possible values, there are other alternative approaches (for instance those of the Michaelis–Menten kind). However, with kinetic based models it is much more difficult to gain insight into system's properties.

Within the general framework defined by the power-law formalism, there are several different approaches to yield a description of the system (Savageau *et al.*, 1987*a*, *b*; Sorribas & Savageau, 1989*a*, *b*; Cascante *et al.*, 1989*a*, *b*). Among them, the S-system representation, eqn (2), has been revealed to be an optimal strategy (Voit & Savageau, 1987; Sorribas & Savageau, 1989*a*, *b*). The S-system representation is obtained after (1) aggregating the different processes affecting a dependent variable X_i into two integrated processes: one accounting for its synthesis and another accounting for its degradation, and (2) deriving a power-law representation for each aggregated process. The resulting S-system representation is:

$$\frac{\mathrm{d}X_i}{\mathrm{d}t} = \alpha_i \prod_{j=1}^{n+m} X_j^{g_{ij}} - \beta_i \prod_{j=1}^{n+m} X_j^{h_{ij}}, \qquad i = 1, \ldots, n. \tag{2}$$

S-system equations have a major advantage: the resulting steady-state equations can be solved explicitly (Savageau, 1970, 1976; see also Sorribas & Savageau, 1989*a*, *c* for numerical examples), which provides a straightforward way of relating the system's behavior with the underlying molecular determinants. This is done by means of logarithmic gains, which evaluate the response of a dependent variable to a change in an independent variable, and parameter sensitivities, which evaluate the local response of a dependent variable to a change in a parameter of the system. In each case, both logarithmic gains and parameter sensitivities are an explicit function of the system's parameters and operating values (Savageau, 1970, 1976), and provide a way for carrying out the mathematical equivalent of a "controlled experiment". This allows for evaluating alternative metabolic designs and to derive very general conclusions concerning their regulatory properties (see some examples in Savageau, 1976; Irvine & Savageau, 1985*a*, *b*). From this point of view, the S-system representation can be considered a landmark concept in the theoretical analysis of metabolic systems.

Besides providing a way for steady-state characterization, the S-system allows for the representation of behavior about the steady-state. Although it is based upon a first-order Taylor expansion, i.e. an approximate rate-law representation, results show accuracy covering several orders of magnitude for changes in an independent variable (Voit & Savageau, 1987; Sorribas & Savageau, 1989*a*, *c*).

Using S-system equations, the steady-state and the system's behavior about its vicinity are fully determined by the operating values of the independent variables and by the parameter values: kinetic orders and rate constants. Hence, two systems which, at an operating point, have identical kinetic orders and rate constants, but different rate equations for the enzymes, will be characterized by the same logarithmic gains and parameter sensitivities. After studying those pathways, we will find no differences and obtain no different information concerning the regulatory properties of both systems. However, it is obvious that those systems having distinct rate equations but identical local and global properties from the S-system point of view, will respond differently to a sufficiently large perturbation. Also, both local and global properties will show a disparate evolution. This can cause some trouble when it comes to the proper characterization of those situations. For properly discriminating both systems, experimental determination of second-order derivatives is needed. This leads us to consider the use of S-system equations that include second-order Taylor terms as a new representation for studying metabolic pathways.

Thus, in this paper we shall analyze the extension of the power-law formalism which results from considering the second-order terms of the Taylor series expansion. Our purpose is: (1) to show that the resulting S-system that includes second-order Taylor coefficients is able to discriminate between the behavior of systems that appear to be equivalent in the approach based on first-order coefficients, (2) to show that the S-system that includes second-order Taylor coefficients yields better accuracy in following the dynamic response and (3) to explore the advantages and disadvantages of using S-systems equations that include second-order Taylor coefficients when it comes to steady-state analysis.

Theory

EXTENSION OF THE POWER-LAW FORMALISM BY USING SECOND-ORDER DERIVATIVES

The power-law representation of a rate law results from a Taylor series expansion in a log–log space after retaining the linear terms (Savageau, 1969*b*, 1970). As discussed in the Introduction, this leads to a useful representation if we sacrifice some accuracy as we move away from the operating point. In the search for a more accurate tool for modeling the actual behavior of a dynamic process, the use of higher terms in the Taylor series seems to provide a natural approach.

In order to derive this power-law based on second-order Taylor coefficients, let us consider any process V_i whose rate depends on $X_1, \ldots, X_{n+m}$ (metabolites, external effectors, enzyme concentration, etc). At an operating point $X_{1(0)}, \ldots, X_{n+m(0)}$ the Taylor series expansion of V_i, in log–log space, can be written:

$$\ln(V_i) = \ln(V_{i0}) + g_i^T(y - y_0) + \frac{1}{2!}(y - y_0)^T g_i'(y - y_0), \tag{3}$$

after retaining the second-order terms.

In this expression $y_i = \ln(X_i)$. The elements of vector g_i are the kinetic orders identified in the power-law formalism, $g_{ij} = [\partial \ln(V_i)/\partial \ln(X_j)]_0$, and the elements of matrix $\mathbf{g}'_i$ are their first derivatives with respect to the different metabolites affecting V_i, i.e.:

$$\mathbf{g}'_{ij,k} = \frac{\partial}{\partial \ln(X_k)}\left(\frac{\partial \ln(V_i)}{\partial \ln(X_j)}\right) = \frac{\partial g_{ij}}{\partial \ln(X_k)}, \tag{4}$$

in each case, the derivatives are evaluated at the operating point. Thus, both g_{ij} and $\mathbf{g}'_{ij,k}$ are local properties, in the sense that they are characteristic features of each individual process at a given point.

Equation (3) can be rewritten as:

$$V_i^+ = V_{i0}^+ \prod_{j=1}^{n+m} \left(\frac{X_j}{X_{j0}}\right)^{G_{ij}} \tag{5}$$

with:

$$G_{ij} = g_{ij} + \frac{1}{2!} \prod_{k=1}^{n+m} g'_{ij,k}(y_k - y_{k0}).$$

Hence, after aggregating individual processes into V_i^+ (production term) and V_i^- (degradation term) (Sorribas & Savageau, 1989*a*, *c*), it is possible to build a mathematical model for a given system using eqn (5) as an approximation for each aggregate rate. This procedure leads to the S-System representation that includes second-order Taylor coefficients:

$$\frac{\mathrm{d}X_i}{\mathrm{d}t} = V_{i0}^+ \prod_{j=1}^{n+m} \left(\frac{X_j}{X_{j0}}\right)^{G_{ij}} - V_{i0}^- \prod_{j=1}^{n+m} \left(\frac{X_j}{X_{j0}}\right)^{H_{ij}}, \tag{6}$$

with H_{ij} in the expansion of the degradation term of X_i analogous to G_{ij} in the expansion of the production term [eqn (5)].

The resulting representation, once parameters g_{ij} and $\mathbf{g}'_{ij,k}$ have been obtained, can be used to predict the system's dynamic behavior and to characterize steady-state properties numerically.

STEADY-STATE PREDICTION USING S-SYSTEM EQUATIONS THAT INCLUDE SECOND-ORDER TAYLOR COEFFICIENTS

If we focus on the changes of independent metabolites, logarithmic gains measure the resulting response of the system at the operating point, and they can be related analytically to kinetic orders in the S-system representation based on first-order coefficients by means of the steady-state solution of the system's equations (Savageau, 1969*b*, 1976; see also Savageau & Sorribas, 1990 for a general discussion). In the S-system equations that include second-order Taylor coefficients, we loose this ability as it is impossible to solve the steady-state equations analytically. This is a limitation when discriminating between systems having the same characterization in the classical S-system representation. However, we can devise a way to obtain the steady-state solution numerically, and, thus, predict new steady-state values after a perturbation.

If we consider that the steady-state values of dependent metabolites are a function of independent metabolites, we can use second-order Taylor series to write an expression relating local systemic responses to predicted steady-state values:

$$y_i = y_{i0} + \boldsymbol{L}(\boldsymbol{X}_i, \boldsymbol{X}_j)(y-y_0)_i + \tfrac{1}{2}(y-y_0)_i^T \mathbf{L}'(\mathbf{L}(\mathbf{X}_i, \mathbf{X}_j), \mathbf{X}_k)(y-y_0)_l$$
$$i = 1, \ldots, n \qquad (7)$$

where the elements of $\boldsymbol{L}(\boldsymbol{X}_i, \boldsymbol{X}_j)$ are $L_{ij} = (\partial y_i/\partial y_j)_0$ for $j = (n+1), \ldots, (n+m)$ and the elements of $\mathbf{L}'(\mathbf{L}(\mathbf{X}_i, \mathbf{X}_j), \mathbf{X}_k)$ are:

$$L'_{ij,k} = \frac{\partial}{\partial y_k}\left(\frac{\partial y_i}{\partial y_j}\right) = \frac{\partial L_{ij}}{\partial y_k}, \qquad k = (n+1), \ldots, (n+m). \qquad (8)$$

We can use eqn (7) to predict steady-state changes from measurements of L_{ij} and $L'_{ij,k}$ at the operating point. First-order coefficients, can be calculated experimentally by means of different procedures. These are described, with applications, in several papers and will not be discussed here (Savageau, 1969*b*; 1976; Voit & Savageau, 1982; Groen *et al.*, 1982, 1986; Mazat *et al.*, 1986; Torres *et al.*, 1986, 1988*a*, *b*; Wanders *et al.*, 1983, 1984). The second-order coefficients can be obtained by computing logarithmic gains at each perturbed steady-state. Then a plot of these first-order coefficients vs. ln ([enzyme] or [effector]) gives the corresponding second-order coefficient. However, in this case, the experimental effort needed to obtain the necessary measures goes up as the square of the number of X variables. Moreover, this requires that the original data be sufficiently accurate. When accuracy is insufficient, the noise will prevent accurate determination of the second-order coefficients. This is a serious difficulty that will prevent one for going further with this type of approach, say to third or fourth-order terms.

In the result section, we shall follow this approach in the numerical computation of L_{ij} and $L'_{ij,k}$ for steady-state predictions by using eqn (7).

Results

REFERENCE SYSTEM

In order to explore the utility in the characterization of the behavior of a system of the S-system equations that include second-order Taylor coefficients, we shall define a reference system using kinetic rate-laws (Fig. 1). This is only for convenience in generating the data we wish to analyze. Hence, simulated behavior from the

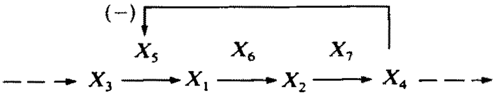

FIG. 1. Metabolic pathway used as an example for comparing the different strategies of mathematical representation (see text).

kinetic rate-laws shall be considered our reality for comparisons on both S-system approaches. All the necessary S-system parameters shall be computed from these simulations as they were experimental results.

In this simple system, X_1 and X_2 are internal metabolites. X_3 is a source metabolite and X_4 is a sink metabolite, both held at a constant amount by the surroundings of the system. X_5, X_6 and X_7 are enzymes catalyzing each reaction. In order to cover different situations, we shall consider two cases:

Case (a) All enzymes follow Michaelian irreversible rate laws with competitive inhibition by their reaction product. The first enzyme also is competitively inhibited by the end product (X_4) [$n_H = 1$ in eqn (9)].

Case (b) The same as (a), but considering that the first enzyme is modulated allosterically by X_4 [$n_H = 4$ in eqn (9)].

The assumed rate-laws are:

$$V_1 = \frac{V_1 X_3}{K_{M_1}\left[1 + \frac{X_1}{K_{I_1}} + \left(\frac{X_4}{S_{0\cdot5}}\right)^{n_H}\right] + X_3} \tag{9}$$

$$V_2 = \frac{V_2 X_1}{K_{M_2}\left(1 + \frac{X_2}{K_{I_2}}\right) + X_1} \tag{10}$$

$$V_3 = \frac{V_3 X_2}{K_{M_3}\left(1 + \frac{X_4}{K_{I_3}}\right) + X_2}. \tag{11}$$

The parameter values assumed for these rate equations in cases (a) and (b) appear in Table 1. These have been chosen to provide the same S-system representation, when using the first-order Taylor coefficients, for both cases (a) and (b). Hence, using the S-system based on a first-order Taylor series will give us the same predictions for both situations.

COMPUTING PARAMETERS FOR S-SYSTEM EQUATIONS

First-order kinetic orders were computed analytically from eqns (9–11) using the values of the parameters in Table 1. The corresponding logarithmic gains were

TABLE 1

Parameter values for the kinetic rate laws

	V	K_m (mM)	K_I (mM)	$s_{0\cdot5}^{nH}$ (mM)	V	K_m (mM)	K_I (mM)	V	K_m (mM)	K_I (mM)
$n_H = 1$	1	0·5	1	1	1·35	1	1·5	1·11	1	1
$n_H = 4$	1	0·875	1·75	7	1·35	1	1·5	1·11	1	1

The values of the concentrations of X_3, X_1, X_2 and X_4 at the chosen operating points for deriving the system representation were 1, 0·8, 1·2 and 1 mM, respectively. Under these conditions the steady-state flux was 0·416 mM sec^{-1}.

calculated from S-system equations using the habitual procedure. Numerical values obtained are coincident both in case (a) $n_H = 1$, and (b) $n_H = 4$ (Tables 2 and 3). Kinetic-order derivatives ($g'_{ij,k}$) were also calculated analytically from kinetic rate-laws. Values of $L'_{ij,k}$ for eqn (7) were calculated as indicated in the Theory section from simulated experiments. Values obtained for cases (a) and (b) appear in Tables 4 and 5.

PREDICTION OF NEW STEADY-STATES

We have studied the values of the dependent variables of the system at the new steady-states reached after different perturbations in X_4 concentration. The perturbations in X_4 tested vary within 50% below and above the operating value. In order to obtain the reference values of the dependent variables of the system (flux, $[X_1]$, and $[X_2]$) at each new steady-state, we have computed the asymptotic value of the corresponding time-course by using kinetic rate laws [eqn (9–11)]. These values have been compared with the values predicted using the S-system based on first-order coefficients [steady-state solution of eqn (2)] or the steady-state equations that include second-order coefficients [eqn (7)] (Fig. 2).

TABLE 2

First-order kinetic orders for cases (a) and (b). Percentage change in the rate-laws of the system in response to a % change in concentration of metabolites. Each term corresponds to $g_{ij} = \partial \ln (V_i^+)/\partial \ln (X_j)$, *or* h_{ij} *for* V_i^-. V_i^+ *refers to the net synthesis of* X_i *and* V_i^- *and* V_i^- *to its net degradation*

	Metabolite concentrations			
Rate-laws	X_1	X_2	X_3	X_4
V_1^+	−0·166	0	0·583	−0·208
$V_2^+ (V_1^-)$	0·692	−0·308	0	0
$V_3^+ (V_2^-)$	0	0·625	0	−0·312

TABLE 3

Logarithmic gains. Percentage change in the dependent variables in response to a % change in the independent variable X_4. *Each term corresponds to* $L(X, X_4) = \partial \ln (X)/\partial \ln (X_4)$, $(X = $ *flux*, $X_1, X_2)$

	Independent variable
Dependent variable	X_4
Flux	−0·180
X_1	−0·166
X_2	0·211

TABLE 4

Kinetic order derivatives for cases (a) and (b). The change in the first-order kinetic orders of the system in response to a % change in concentration of metabolites. Each term corresponds to $g'_{ij,k} = \partial g_{ij}/\partial \ln (X_k)$ *or* $h'_{ij,k}$ *for* h_{ij}

	Metabolite concentrations			
Kinetic orders	X_1	X_2	X_3	X_4
g_{11}	−0·139	0	0·0694	0·0347
g_{12}	0	0	0	0
g_{13}	0·0694	0	−0·243	0·0870
g_{14}	0·0347	0	0·0870	−0·165†/−0·790‡
h_{11}	−0·213	0·0947	0	0
h_{12}	0·0947	−0·213	0	0
h_{13}	0	0	0	0
h_{14}	0	0	0	0
h_{21}	0	0	0	0
h_{22}	0	−0·234	0	0·117
h_{23}	0	0	0	0
h_{24}	0	0·117	0	−0·215

† $n_H = 1$ and ‡ $n_H = 4$.

TABLE 5

Logarithmic gains derivatives. The change in the first-order logarithmic gain in response to a % change in the independent variable X_4. *Each term corresponds to* $L'[L(X, X_4), X_4] = \partial L(X, X_4)/\partial \ln (X_4)$ $(X =$ *flux*, $X_1, X_2)$

	Independent variable	
	X_4	
Logarithmic gain	$(n_H = 1)$	$(n_H = 4)$
$L(J, X_4)$	−0·152	−0·612
$L(X_1, X_4)$	−0·171	−1·16
$L(X_2, X_4)$	0·0384	−0·697

Results show both first- and second-order approaches to be accurate when $n_H = 1$ (see Table 6 and Fig. 2). In this simulated experiment, predictions based on the use of second-order coefficients [eqn (7)] are closest to the observed behavior after integration of the kinetic equations. The observed accuracy range is in agreement with previous published results for the S-system based on the first-order coefficients (Voit & Savageau, 1987; Sorribas & Savageau, 1989*a*, *c*).

A different behavior of the steady-state curve is obtained if $n_H = 4$ [curves labeled as (□) in Fig. 2]. In this case, however, the first-order based S-system predicts no

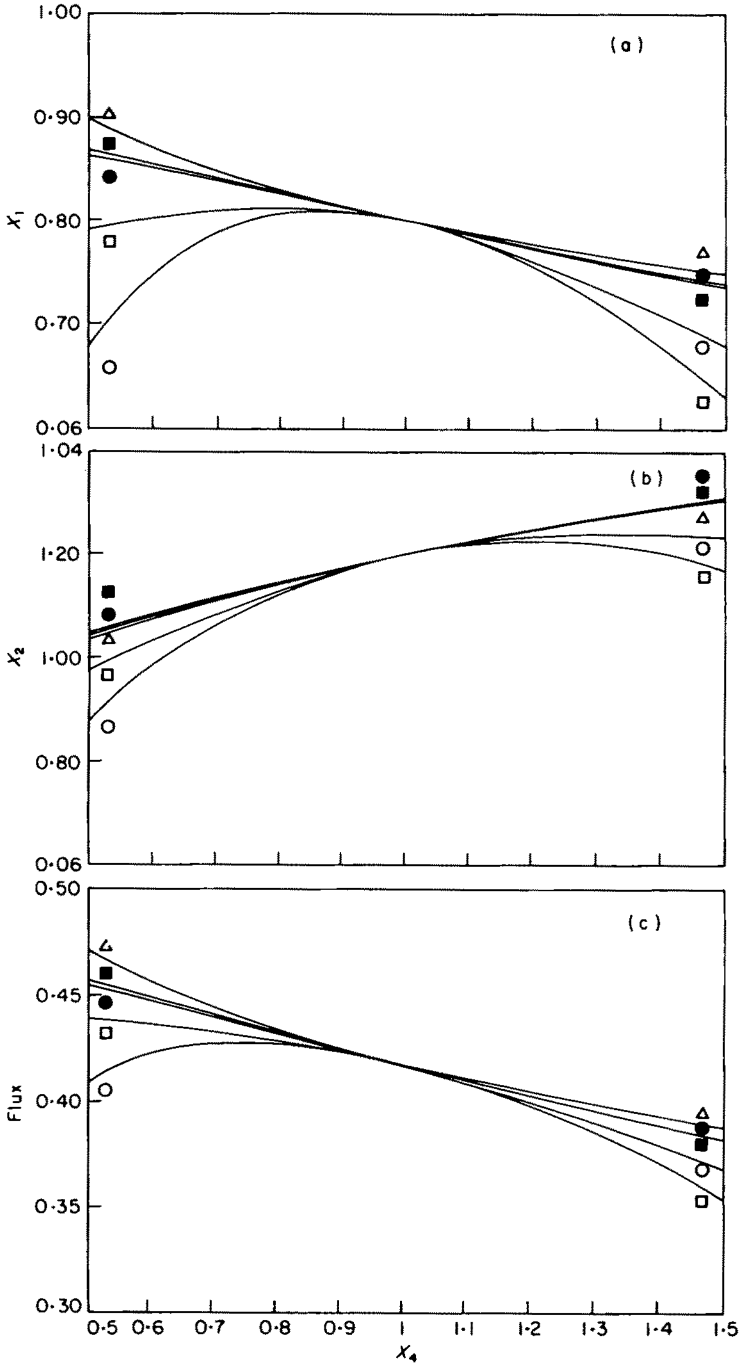

FIG. 2. Steady-state behavior of the system in Fig. 1 in response to changes in X_4. (a) X_1; (b) X_2; (c) flux. Reference behavior obtained from kinetic equations is labeled (■) ($n_H = 1$) and (□) ($n_H = 4$). Behavior obtained from the S-system based on the first-order Taylor coefficients are labeled (△). Results obtained from the S-system equations that include second-order Taylor coefficients are labelled as (●) ($n_H = 1$) and (○) ($n_H = 4$).

TABLE 6

Accuracy of the predictions of first- and second-order approaches. Range of values of the independent variable X_4 (inside the interval of perturbations in X_4 tested: 0·5–1·5) leading to an error ≤5% in the value of the dependent variable predicted using first- or second-order approaches

Dependent variable		X_4 range			
		$n_H = 1$		$n_H = 4$	
		First-order	Second-order	First-order	Second-order
J	Range	0·50–1·50	0·50–1·50	0·59–1·38	0·57–1·50
	error (%)	3·06–1·30	0·65–0·00	5·00–5·00	5·00–3·90
X_1	Range	0·50–1·50	0·50–1·50	0·69–1·27	0·63–1·43
	error (%)	3·30–1·62	0·80–0·14	5·00–5·00	5·00–5·00
X_2	Range	0·50–1·50	0·50–1·50	0·55–1·34	0·59–1·49
	error (%)	1·33–0·23	0·47–0·08	5·00–5·00	5·00–5·00

different behavior as its parameters have the same values in both situations [curve labeled as (△) in Fig. 2]. Hence, predictions using this approach have a more limited range of accuracy (see Table 6). On the contrary, the inclusion of second-order Taylor coefficients result in the ability of giving an appropriate prediction of this case. The obtained behavior from the second-order approach [curve labeled as (○) in Fig. 2] shows good accuracy with the simulated experimental data (see Table 6). As expected, when we consider a larger perturbation, this representation looses accuracy. Under the conditions we studied, this is particularly critical for X_1 and perturbations of X_4 below the operating point. In general, when one explores accuracy using the second-order approach a similar behavior is found: a relative wide range of high accuracy and a quick loss of accuracy outside this range (results not shown).

SYSTEM'S BEHAVIOR

The reference dynamic behavior of the pathway of Fig. 1 (for $n_H = 1$ or $n_H = 4$) has been studied when the concentration of the external effector X_4 is changed within a range of 50% above and below its operating value. The reference behavior of this system was obtained by solving numerically the differential equations built from eqns (9–11) by using a modified Runge–Kutta method (Franco & Canela, 1984). This behavior has been compared with the predictions made by using both the S-system equations based on first-order Taylor coefficients [eqn (2) with the corresponding parameter values of Table 2] and with the S-system equations that include second-order Taylor coefficients [eqn (5) with the corresponding parameter values of Table 3]. Results are shown in Fig. 3 for the extreme values of X_4 considered.

As it happened in the steady-state predictions, when $n_H = 1$ both first- and second-order approaches lead to a good prediction. However, it should be noted

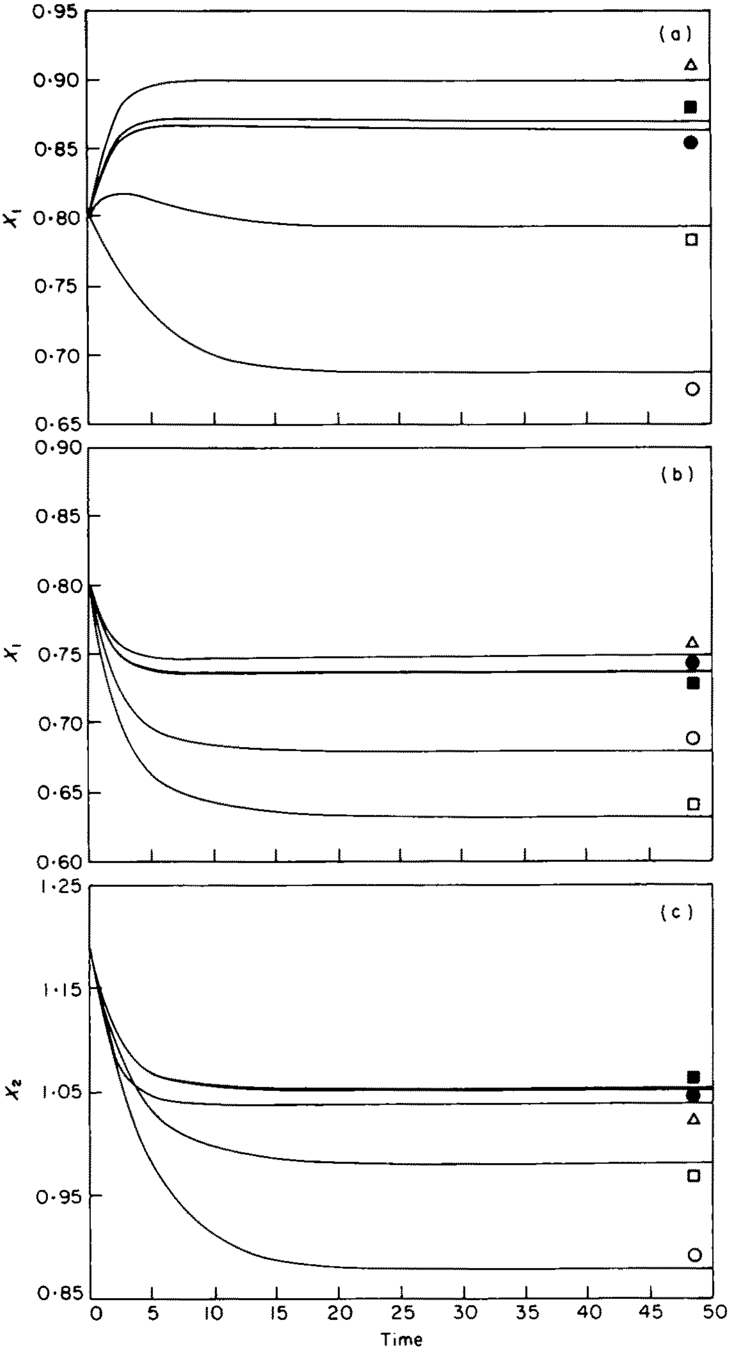

FIG. 3. Dynamic behavior of the system in Fig. 1 in response to a perturbation in X_4. The considered perturbation is $X_4 = 0{\cdot}5$ for (a), (c) and (e), and $X_4 = 1{\cdot}5$ for (b), (d) and (f). These perturbations correspond to the extreme values considered in Fig. 2. Curves are identified by the same labels as in Fig. 2.

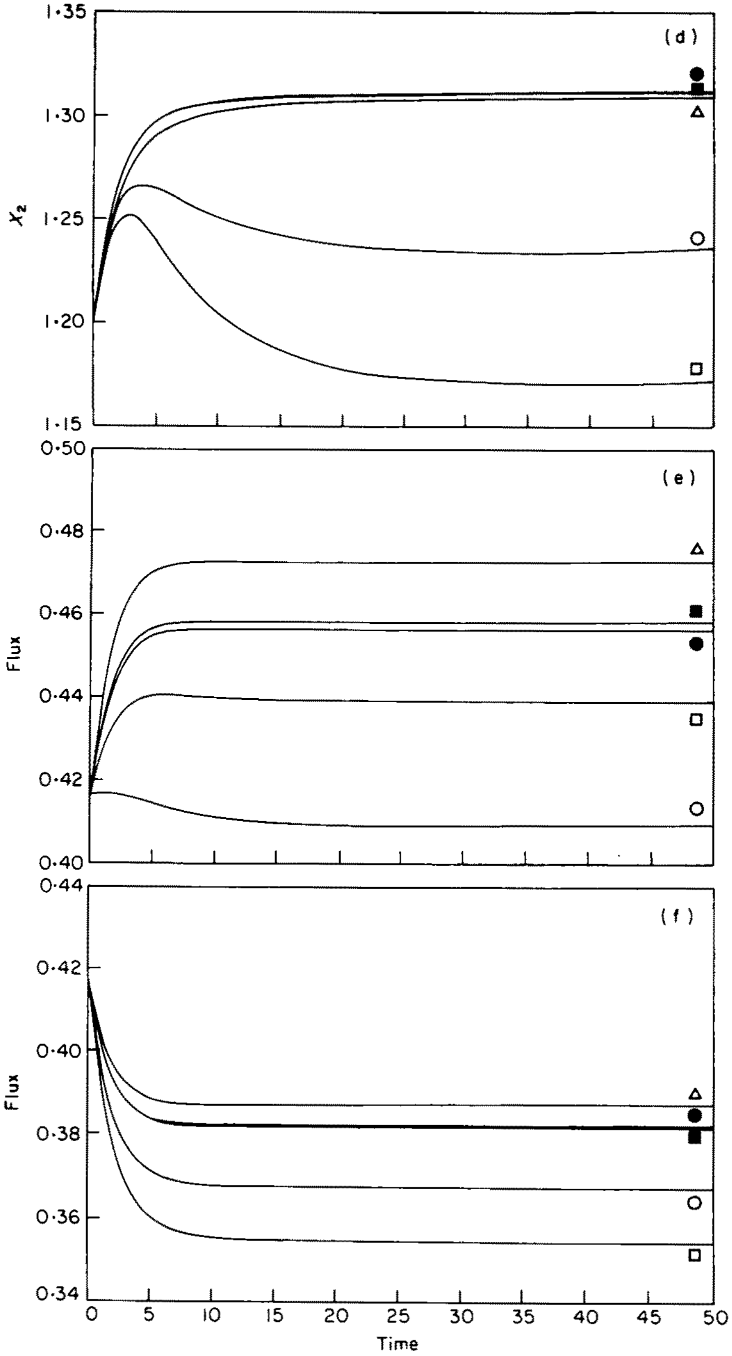

FIG. 3—continued

that second-order equations produce a more accurate representation that is almost indistinguishable from that obtained from the kinetic equations [Fig. 3(b), (c), (d) and (f)].

When kinetic equations include more complicated mechanisms, which in our reference system corresponds to $n_H = 4$, both approaches run into trouble when attempting to predict accurately the transient response that results from a large perturbation in X_4. Qualitative predictions are better when the perturbation is placed above the operating point [Fig. 3(b), (d) and (f)]. These predictions are worse when the perturbation is placed below the operating point [Fig. 3(a), (c) and (e)]. It should be noted that the second-order approach can produce a very bad prediction in some cases [Fig. 3(a) and (c)]. However, we are considering the extreme values of X_4 in Fig. 2. and, thus, the considered transient responses in Fig. 3 represent the worst situation. When perturbation in X_4 is placed within the range leading to an accurate steady-state prediction, results become much more accurate by using second-order equations (results not shown). This is in agreement with previously published results exploring accuracy for the S-system representation based on first-order Taylor coefficients (Voit & Savageau, 1987; Sorribas & Savageau, 1989*a*, *c*).

Discussion

Since its introduction in the late 1960s, the power-law formalism has produced useful results in the analysis of metabolic pathways. Most specially, the S-system variant of this approach has become a well-developed tool able to both characterize the steady-state and predict the dynamic behavior of the system around a reference operating point. Furthermore, it provides a way for accurate comparison of different metabolic designs, which allows understanding of their advantage according to several criteria. This ability makes the S-system representation a valuable tool for the analysis of biochemical pathways.

In search of a new development within this methodology, we have explored an extension of the former power-law to produce an S-system representation that includes second-order Taylor coefficients. From the results presented in this paper, we suggest that this new representation extends the range for which the dynamic behavior of the system can be predicted accurately. Moreover, we have shown that the measure of second-order derivates allows for discriminating between systems that yield the same first-order derivative terms.

Hence, when the main interest is modeling the system in order to make quantitative predictions of changes in response to perturbations, the S-system equations that include second-order Taylor terms shall provide a more accurate representation after some extra experimental effort in accurately obtaining these coefficients. In practice, however, accuracy problems arise and prevent from going further on this kind of approach, say to third- and fourth-order coefficients.

It is important to recall that power-law based models provide an alternative to the classic kinetic based models for representing a system. As results from the accumulated experience in using this methodology, models based on the power-law formalism should become a standard for several reasons already stated in the Introduction: (1) they are simple; (2) they are systematic; and (3) they provide a useful representation of the system. In this sense, experimental effort should focus on measurements of the intact system rather than of isolated reactions. After paying a small toll on accuracy for using an approximate rate-law, we shall gain further understanding of the integrated behavior of our system of interest.

If we focus on properly understanding this class of problems, and despite the advantages discussed above when modeling dynamic behavior, the S-system representation based on second-order Taylor coefficients lacks the ability to evaluate the system analytically. This is a fundamental restriction if we are interested in theoretically analyzing a system to gain a proper understanding of its regulatory properties. In this sense, the S-system representation based on first-order Taylor coefficients is superior. Moreover, when it comes to consider the behavior of a metabolic system *in situ*, it is clear that both S-system representations are able of giving an accurate prediction, within a reasonable range of error (5%) when compared to experimental measurements, for quite large changes of the independents variables (see Table 6). This suggest that, although S-systems based on second-order Taylor coefficients do improve the accuracy, for practical purposes the S-system equations based on first-order coefficients should be considered as a more convenient representation.

We thank E. Meléndez-Hevia, N. Torres, E. O. Voit, D. H. Irvine and M. A. Savageau for their helpful discussions and suggestions for improving the manuscript. We also thank the Comissió Interdepartmental de Recerca i Innovació Tecnològica de Generalitat de Catalunya for their financial support which enabled Marta Cascante to stay in The University of Michigan. Albert Sorribas was partially supported by Grant of the University of Barcelona (Ajuda a Grups de Recerca de Nova Formació, 1990).

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