The Tricarboxylic Acid Cycle in *Dictyostelium discoideum*

I. FORMULATION OF ALTERNATIVE KINETIC REPRESENTATIONS* 

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Enzyme systems within living cells have recently been shown to be highly ordered structures that violate classic assumptions of the Michaelis-Menten formalism, which originally was developed for the characterization of isolated reactions in *vitro*. This evidence suggests that a thorough examination of alternative kinetic formalisms for integrated biochemical systems is in order. The purpose of this series of papers is to assess the utility of an alternative power-law formalism by carrying out a detailed comparative analysis of a relatively large, representative system — the tricarboxylic acid cycle of *Dictyostelium discoideum*. This system was chosen because considerable experimental information already has been synthesized into a detailed kinetic model of the intact system. In this first paper, we set the stage for subsequent analysis within the framework of the power-law formalism: we review the underlying theory, emphasizing recent developments, formulate the model in terms that are convenient for the analysis to follow, and develop the system representation in both the Michaelis-Menten and power-law forms. In the second paper (Shiraiishi, F., and Savageau, M. A. (1992) *J. Biol. Chem.* 267, 22919-22925), these alternative representations are shown to be internally consistent and locally equivalent. The third paper (Shiraiishi, F., and Savageau, M. A. (1992) *J. Biol. Chem.* 267, 22926-22933) provides a complete analysis of the steady state behavior and also treats the dynamic behavior of the model.

The canons of good enzymological practice were developed to guide the study of kinetics in *vitro*. They have proved to be remarkably useful for the study of isolated reaction mechanisms, but they are often highly inappropriate for the study of integrated biochemical systems in *vivo* (1-3). For example, the assumptions of the Michaelis-Menten formalism (4-6) are violated by enzyme-enzyme interactions in *vitro*, which suggest that there may be problems in using this formalism to characterize the behavior of enzymes within integrated biochemical systems (3). A rigorous evaluation of these problems is in order with thorough consideration being given to alternative formalisms. One of these alternatives is the power-law formalism in which the rates of reactions are described by products of power-law functions (7). This formalism was developed specifically for the characterization of integrated biochemical systems in *vivo*.

The purpose of this series of papers is to assess the utility of the power-law formalism by carrying out a detailed analysis of a relatively large, representative system — the tricarboxylic acid cycle of *Dictyostelium discoideum*. It was chosen because considerable experimental information already has been synthesized into a detailed kinetic model of the intact system by Wright *et al.* (8). The model can be used to generate data that serve as a convenient reference for comparative purposes.

In this first paper, we set the stage for subsequent analysis within the framework of the power-law formalism. First, we review the underlying theory, emphasizing recent developments. Second, we use the data of Wright *et al.* (8) to formulate the model in terms that are convenient for the analysis to follow. Finally, we develop the system representation in both the Michaelis-Menten and power-law forms.

In the second paper (42), these alternative representations are shown to be internally consistent and locally equivalent. The second paper also provides a detailed and systematic analysis of model robustness. The methods employed for the analysis take advantage of the regular mathematical structure exhibited by the power-law formalism. The third paper (43) provides a complete analysis of the steady state behavior based on the power-law representation. These results are compared with those obtained by a similar analysis based on the Michaelis-Menten representation. Finally, the third paper examines the dynamic behavior of the model.

**BIOCHEMICAL SYSTEMS THEORY**

The proposal for a biochemical systems theory based on the power-law formalism (9) grew out of concepts from classical network and system-sensitivity theories (10). At the level of rate laws for individual reactions, the exponents in the power-law functions are the kinetic orders of the reaction in the usual sense. However, they can take on values that are fractional as well as integer, negative as well as positive, and such exponents exist for all variables that influence the system, not just the usual reactant and modifier concentrations. It is clear from the application of this formalism at the chemical level that it includes traditional mass-action kinetics and in turn Michaelis-Menten kinetics as special cases when the appropriate restrictive assumptions are imposed (Fig. 1). At the level of the intact system, the local steady state behavior was solved within the power-law formalism by a judicious aggregation of flux. Shortly thereafter, explicit relationships between systemic and molecular properties were derived (11); *logarithmic-gain factors* that characterize the
global behavior of an intact system were shown to depend only on the apparent kinetic orders that characterize the underlying molecular processes. Subsequently, two different lines of evidence have developed that suggest the relevance of this power-law formalism for intact biochemical systems.

From a top-down perspective, it has been shown (12, 13) that this formalism is consistent with the macroscopic growth laws of intact organisms (e.g. see Refs. 14–16) and with the allometric relationships that quantitatively characterize relative growth among the parts of organizationally complex biological systems (e.g. see Refs. 14, 17, and 18). This is not the case for the other two formalisms commonly used in biochemistry—the linear formalism and the Michaelis-Menten formalism. The linear formalism implies linear relationships among the constituents of a system in quasi-steady state, which is inconsistent with the wealth of experimental evidence showing that these relationships are nonlinear in most cases. The case of the Michaelis-Menten formalism is more problematic. Within this formalism there is no known solution in terms of elementary mathematical functions for an arbitrary system of reactions, so it is difficult to determine whether or not this formalism is consistent with the experimentally observed data. However, it is possible to deduce the systemic behavior of simple specific systems involving a few reactions and find examples in which the elements do not exhibit allometric relationships. So in general, the Michaelis-Menten formalism is not consistent with allometric relationships at the system level. As indicated below, there are other reasons to suspect that the Michaelis-Menten formalism is inappropriate for representing the kinetics of reactions in vivo.

From the bottom-up perspective, there is now solid experimental evidence for the fractional kinetic orders found in the power-law formalism (e.g. Refs. 19–22). Kinetic orders typically have positive integer values in traditional chemical kinetics, where reactions are assumed to occur in dilute homogeneous solutions. These integers add up to the molecularity of the reaction. The concepts of kinetic order and molecularity are distinct, although they often are confused and considered to be equivalent because of this relationship in dilute homogeneous solutions. In contrast to the traditional view, these recent studies have shown that the kinetic order of a reaction with respect to a given reactant is a function of the geometry within which the reaction takes place. In the simplest case of reactions occurring in a homogeneous solution within a three-dimensional volume, the kinetic order is identical with the number of molecules entering into the reaction. When reactions occur on a two-dimensional surface, the kinetic order is larger, and when they are restricted to a one-dimensional channel, it becomes even larger still. For example, a bimolecular reaction in a three-dimensional homogeneous space has a kinetic order of 2, but the same reaction on a two-dimensional lattice has a kinetic order of 2.46 and on a one-dimensional lattice it has a kinetic order of 3 (19).

Reactions in vivo often occur on membranes or in channels. One of the best documented examples is provided by recent work on the two reactions of the tryptophan synthase complex of Salmonella typhimurium (23, 24). The product of the first reaction and the substrate of the second is indole. This intermediate passes from one catalytic site to the next through a tunnel whose length is about 4 times that of the indole.
molecule. In such cases, the kinetic orders can be expected to exhibit fractional values that are larger than the number of molecules entering into the reaction, and the forms of the rate laws may well differ from those obtained by assuming homogeneous solutions in three-dimensional volumes. The power-law formalism provides the context for assessing the importance of fractal kinetics in the quantitative characterization of such systems.

Previous research on this power-law formalism has focused on a number of issues, including the representation of nonlinear rate laws for small local variations (25) as well as large global variations (26), analytical (26) and numerical (27) methodology, and the aggregation of fluxes and pools in nonlinear systems consisting of branching reactions (28) and small networks of reactions (29, 30). For an overall review of the field see Voit (31). There also have been numerous applications to biochemical systems of modest size, but until recently there have been few attempts to analyze relatively large systems, like the tricarboxylic acid cycle.

One of the important issues concerning the utility of the power-law formalism is the accuracy with which it represents the real system. Early analyses showed that the local power-law representation of isolated reactions characterized by the classical Michaelis-Menten equation is accurate for a 2- to 3-fold range of variation in substrate concentration (9, 32). When the power-law representation of converging and diverging reactions was compared with the classical representation for hyperbolic and sigmoid rate laws, it was found to be accurate for a minimum range of about 4- to 5-fold in reactant and modifier concentrations (28). More recently, traditional chemical kinetics and power-law kinetics were used to represent a small network of four reactions involving enzyme-modifier interactions and the channeling of intermediates. The behavior was analyzed using each representation, and agreement was found over an average range of 20-fold, which is greater than the average range of 5-fold found for biochemical variables measured in the clinical setting (29). This evidence suggests that the local power-law representation is sufficiently accurate to represent the behavior of many biochemical systems over a physiologically appropriate range. Moreover, as the complexity of the systems being examined has increased, from single reactions to pairs of converging or diverging reactions to small networks of reactions, the accuracy has increased on average.

This leads us to hypothesize that accuracy may well be even better for relatively large biochemical systems. An analogy is provided by the kinetic theory of gases. In this theory, the behavior of small numbers of molecules is poorly represented, but the macroscopic behavior of large numbers of molecules is accurately represented. The microscopic errors tend to cancel when one averages over large numbers. Indeed, we have shown that cancellation of errors with opposite signs does occur when individual reactions are combined into small systems (28, 29). We shall test this hypothesis further in a subsequent paper.

MODEL OF THE TRICARBOXYLIC ACID CYCLE

The tricarboxylic acid cycle plays an essential role in the aerobic metabolism of Dictyostelium (33). It decomposes pyruvate to water and CO₂ via acetyl-CoA and simultaneously produces ATP with high efficiency. During growth of the organism in the single-cell form, ingested protein is broken down into amino acids that feed the cycle and provide building blocks for anabolism; with the onset of starvation, cells aggregate and undergo development into the multicellular form, oxidative metabolism changes, and cellular protein is used mainly to fuel the tricarboxylic acid cycle (34-36). The enzymes that constitute this central process of intermediary metabolism have been characterized extensively through a variety of physicochemical studies and detailed steady state kinetic measurements. The elucidation of enzyme mechanism has been the principal object of these studies.

There are relatively few studies aimed at characterizing the operation of the tricarboxylic acid cycle in vivo. The work of Wright et al. (8) is a notable exception. They have proposed a model of the cycle that is based on kinetic studies in vitro and labeling studies in vivo. Their model features amino acid fluxes from protein degradation that enter at several points of the cycle; also various pools are divided into separate compartments according to their role so as to identify most clearly the functional system in the mitochondria (36, 37). Unknown values for parameters in the model were varied until model behavior matched labeling patterns experimentally determined in vivo (37). On the basis of this work, Wright et al. (8) have formulated a more detailed model of the

![Fig. 2. Schematic model of the tricarboxylic acid cycle in D. discoideum. Metabolites are shown in boxes, and reactions are indicated by arrows, irreversible or reversible. Dependent metabolites are numbered from 1-13; independent variables are numbered from 14-39 and 46-50. Enzymes that have been kinetically characterized in vitro have their numbers circled. For simplicity of representation, not all cofactors for each reaction are shown explicitly. However, the cofactor dependencies are given explicitly in the appropriate rate laws (see Equation A1). For further information, see Table I and the text. After Kelly et al. (37) and Wright et al. (8).]
tricarboxylic acid cycle in which the rate laws for the reactions are represented by rational functions within the Michaelis-Menten framework. This model, shown schematically in Fig. 2, is an extension and refinement of the earlier model published by Kelly et al. (37) in that various pools have been either separated or aggregated to better reflect the functional tricarboxylic acid cycle in mitochondria (8).

In any such system there are two classes of variables that must be distinguished: independent variables have values that are determined, independent of other variables, by the environment or by the investigator who can independently modify these by experimental means; all other variables are dependent on the values of the independent variables and are therefore dependent variables. The model in Fig. 2 consists of 13 dependent concentration variables [X_i] to [X_{13}] and 31 independent variables, which include enzyme concentrations [X_{14} to X_{31}], cofactor concentrations [X_{32} to X_{45}], and fixed reservoirs for protein and CO_2 [X_{46} and X_{50}].

The names of the enzymes that catalyze the numbered reactions in Fig. 2 are listed in Table I. Enzymes that have their numbers circled have been isolated, purified, and kinetically characterized in vitro (8). Enzymes 16–18, 28, and 29 catalyze reversible reactions; all others are considered essentially irreversible. It should be noted that concentrations of the cofactors NAD [0.072 mM], CoA [0.100 mM], and NADH [0.180 mM] are assumed to be constant, and that the pools for protein and CO_2 are assumed to have no influence on the reactions of this model.

**SYSTEMIC REPRESENTATION**

**Kirchhoff's Node Laws**—The fundamental equations that characterize the dependent concentrations are given by Kirchhoff's node equations, which also are referred to as mass balance equations. For the model in Fig. 2 they can be written

\[
dX_i/dt = (v_1 + v_{23} + v_{30,2}) - (v_1 + v_{24}) = V_1 - V_{1,-}
\]

\[
dX_2/dt = (v_{12} + v_{27}) - (v_2 + v_{50}) = V_2 - V_{2,-}
\]

\[
dX_3/dt = (v_{30} + v_{56,3}) - (v_{50}) = V_3 - V_{3,-}
\]

\[
dX_4/dt = (v_{40} + v_{50}) - (v_4) = V_4 - V_{4,-}
\]

\[
dX_5/dt = (v_{50} + v_{13,3}) - (v_{50} + v_{50}) = V_5 - V_{5,-}
\]

\[
dX_6/dt = (v_{60} + v_{13,6} + v_{60}) - (v_{60} + v_{60,10} + v_{61,11}) = V_6 - V_{6,-}
\]

\[
dX_7/dt = (v_{70} + v_{70,12} + v_{12} + v_{70} + v_{70,8}) = V_7 - V_{7,-}
\]

\[
dX_8/dt = (v_{80} - v_{80,9}) - (v_{90}) = V_8 - V_{8,-}
\]

\[
dX_9/dt = (v_{90} - v_{90}) = V_9 - V_{9,-}
\]

\[
dX_{10}/dt = (v_{10} + v_{10,10} + v_{10}) - (v_{10} + v_{10,11}) = V_{10} - V_{10,-}
\]

\[
dX_{11}/dt = (v_{11,11} + v_{11,11} + v_{11,11}) - (v_{11} + v_{11,12}) = V_{11} - V_{11,-}
\]

\[
dX_{12}/dt = (v_{12,12} + v_{12,12}) = V_{12} - V_{12,-}
\]

\[
dX_{13}/dt = (v_{13,13} + v_{13,13}) = V_{13} - V_{13,-}
\]

In these equations, \( v_0 \) signifies the rate of utilization of metabolite \( X_i \) for the production of metabolite \( X_j \); it also represents the flux through a reaction. The symbols \( V_i \) and \( V_{i,-} \) represent aggregate flux into and out of the \( X_i \) pool. Note that we have made use of the fact that \( v_{50} = v_{50} \cdot v_{10} = v_{50} \cdot v_{50} \), and \( v_{13,12} = v_{13,12} \), where the third subscript signifies a second parallel reaction between the metabolites indicated by the first two subscripts.

There is a variety of equally valid ways to aggregate the elementary fluxes in Kirchhoff's equations. In the above equations, we have grouped all the positive fluxes together and all the negative fluxes together so as to define aggregate influxes and effluxes. This is possible because the direction of flux through the reversible reactions is unchanged under the conditions examined in this paper; so no fluxes change sign and we are able to use the irreversible strategy for representing the fluxes in the power-law formalism. This is the most common strategy one finds in the literature. If we were to encounter conditions leading to a reversal of flux through any reaction, then we would employ the more appropriate reversible strategy, which avoids any problems with fluxes changing sign (30).

**Michaelis-Menten Representation**—The rate law for each reaction has been determined within the Michaelis-Menten tradition from steady state kinetic measurements, or standard rate laws have been assumed (8). In this formalism, the rate laws are linear functions of enzyme concentration and rational functions of the various reactant and modifier concentrations.

### Table I

**Enzymes of the tricarboxylic acid cycle in Dictyostelium discoideum**

<table>
<thead>
<tr>
<th>Variable number</th>
<th>Reaction*</th>
<th>EC number</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>Glutamate dehydrogenase (GluDH)</td>
<td>1.4.1.2</td>
</tr>
<tr>
<td>15</td>
<td>α-Ketoglutarate dehydrogenase complex (α-KGDH)</td>
<td>1.3.99.1</td>
</tr>
<tr>
<td>16</td>
<td>Succinate dehydrogenase (SDH)</td>
<td>2.8.3.1</td>
</tr>
<tr>
<td>17</td>
<td>Fumarase (Fumarase)</td>
<td>4.2.1.2</td>
</tr>
<tr>
<td>18</td>
<td>Malate dehydrogenase (MDH)</td>
<td>1.1.1.37</td>
</tr>
<tr>
<td>19</td>
<td>Malic enzyme (ME)</td>
<td>1.1.1.40</td>
</tr>
<tr>
<td>20</td>
<td>Asp → Pyr</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>Pyruvate dehydrogenase complex (PDC)</td>
<td>1.2.4.1</td>
</tr>
<tr>
<td>22</td>
<td>Pyruvate dehydrogenase</td>
<td>2.3.1.12</td>
</tr>
<tr>
<td>23</td>
<td>Dihydrolipoil dehydrogenase</td>
<td>1.8.1.4</td>
</tr>
<tr>
<td>24</td>
<td>Asp → Oaa2</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>Citrate synthetase (CS)</td>
<td>4.1.3.7</td>
</tr>
<tr>
<td>26</td>
<td>Aconitase</td>
<td>4.2.1.3</td>
</tr>
<tr>
<td>27</td>
<td>Isocitrate dehydrogenase (IsocDH)</td>
<td>1.1.1.41</td>
</tr>
<tr>
<td>28</td>
<td>Glu → Suc</td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>Aspartate transaminase (AspTA)</td>
<td>2.6.1.1</td>
</tr>
<tr>
<td>30</td>
<td>Alanine transaminase (AIA TA)</td>
<td>2.6.1.2</td>
</tr>
<tr>
<td>31</td>
<td>Oaa1 → Oaa2</td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>Asp → Oaa1</td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>Suc → Glu</td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>Oaa2 → Asp</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>Prot → Asp</td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>Prot → AcCoA</td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>Prot → Suc</td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>Prot → Fum</td>
<td></td>
</tr>
<tr>
<td>39</td>
<td>Prot → Ala</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>Prot → Glu</td>
<td></td>
</tr>
</tbody>
</table>

* See Fig. 2.

\n
### Table II

**Nominal values for the enzyme concentrations**

See text for discussion of nominal enzyme concentration. The units are mM min\(^{-1}\). except in the cases of X_{30}, X_{31}, X_{32}, X_{34}, X_{36}, X_{50}, X_{51}, X_{53}, and X_{33}, where the units are min\(^{-1}\). Data provided by Wright et al. (8).

<table>
<thead>
<tr>
<th>Variable number</th>
<th>Nominal value</th>
</tr>
</thead>
<tbody>
<tr>
<td>X_{14}</td>
<td>9.71D-01</td>
</tr>
<tr>
<td>X_{24}</td>
<td>1.00D-01</td>
</tr>
<tr>
<td>X_{32}</td>
<td>1.00D-00</td>
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<tr>
<td>X_{55}</td>
<td>7.61D+03</td>
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<tr>
<td>X_{64}</td>
<td>8.24D+00</td>
</tr>
<tr>
<td>X_{65}</td>
<td>7.40D+01</td>
</tr>
<tr>
<td>X_{23}</td>
<td>3.15D+00</td>
</tr>
<tr>
<td>X_{34}</td>
<td>8.00D+01</td>
</tr>
<tr>
<td>X_{66}</td>
<td>2.38D+01</td>
</tr>
<tr>
<td>X_{15}</td>
<td>2.57D+01</td>
</tr>
<tr>
<td>X_{56}</td>
<td>2.71D+02</td>
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<tr>
<td>X_{67}</td>
<td>4.60D-01</td>
</tr>
<tr>
<td>X_{16}</td>
<td>7.78D+01</td>
</tr>
<tr>
<td>X_{68}</td>
<td>1.33D+01</td>
</tr>
<tr>
<td>X_{69}</td>
<td>3.60D-01</td>
</tr>
<tr>
<td>X_{17}</td>
<td>3.05D+00</td>
</tr>
<tr>
<td>X_{61}</td>
<td>9.35D+00</td>
</tr>
<tr>
<td>X_{92}</td>
<td>8.00D-02</td>
</tr>
<tr>
<td>X_{20}</td>
<td>1.96D-01</td>
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<tr>
<td>X_{71}</td>
<td>2.67D+01</td>
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<td>X_{29}</td>
<td>4.50D-01</td>
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<td>X_{21}</td>
<td>2.58D+02</td>
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<tr>
<td>X_{72}</td>
<td>8.00D+02</td>
</tr>
<tr>
<td>X_{30}</td>
<td>7.40D+01</td>
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</tbody>
</table>

* See Fig. 2 and Table I for enzyme location and name.

\n
\textsuperscript{a} The values are given in scientific notation; D-01 signifies multiplication by 10\(^{-1}\) in double precision arithmetic; values have been rounded to three significant digits.
(38, 39). Although enzyme concentrations per se have not been represented in this model of the tricarboxylic acid cycle, there is in each case a variable proportional to enzyme concentration that has been experimentally specified. Henceforth, these variables, rather than true enzyme concentrations, will be represented by the symbols $X_{14}$ through $X_{30}$. Their nominal values are given in Table II. One may continue to think of these variables as enzyme concentrations that have had their units suitably converted for this model. The individual rate laws for the Michaelis-Menten representation of the tricarboxylic acid cycle are given for reference in the Appendix.

**Power-Law Representation**—The constellation of definitions, concepts, and methodology that emphasizes the characterization of integrated systems of enzyme-catalyzed reactions within the context of the power-law formalism is referred to as biochemical systems theory (25). Although the power-law formalism could be used to describe the tricarboxylic acid cycle at the level of elementary chemical kinetic steps, this would produce an overly complex model for which there would be no experimental data to determine the parameter values. At any higher level of representation, such as the level of individual enzyme-catalyzed reactions, one must deal with the issue of aggregation (28). Rate laws in the Michaelis-Menten formalism represent the summation of elementary fluxes into one aggregate flux through the reaction. Alternatively, rate laws within this formalism could be derived with summation of elementary fluxes into one aggregate flux through the substrate or product pool. Rate laws in the power-law formalism have been derived by both of these aggregation strategies. Detailed comparisons have shown that aggregation of elementary fluxes through pools provides a more mathematically tractable representation, which also is more accurate in most cases (28, 29, 40). For these reasons, and others (41), we have chosen to aggregate elementary fluxes through pools, which leads to the so-called S-system representation within biochemical systems theory.

Each rate law in the power-law form is composed of a product of power-law functions, one for each variable that affects the rate of the process. The values of the parameters are easily determined (25). The values for the exponents are obtained directly from experimental data, or from any mathematical representation of such data. The slope in a log-log plot of rate versus concentration data gives the corresponding kinetic order directly; appropriate partial differentiation of the corresponding mathematical representation gives the same kinetic order. The value of the rate constant is then easily determined with any convenient set of values for the rate, the concentrations, and the kinetic orders that have just been determined. Since the Michaelis-Menten representation of the kinetic data is available for the tricarboxylic acid cycle, we have used the second approach to determine the kinetic orders and rate constants for the aggregate rate laws. The aggregate rate laws for the S-system representation of the tricarboxylic acid cycle are given for reference in the Appendix.

These aggregate rate laws can be obtained from experiments performed with mixtures of the relevant enzymes, or from the mathematical representation of the rate laws for such mixtures. For example, the Michaelis-Menten representation of the aggregate rate law for the two enzymes removing succinate from the pool $X_{11}$ is

$$V_{-11} = X_{12}(X_{11} + X_{16}(X_{11} - 0.100X_{15}))/0.100 + X_{15} + 0.100X_{12}$$  \hspace{1cm} (Eq. 2)

The corresponding power-law representation about the nominal steady state with $X_{12}$, $X_{16}$, and $X_{32}$ values of 0.801, 0.0400, 3.15, and 1.00 is

$$V_{-11} = 1.54X_{12}^{0.317}X_{16}^{-0.00733}X_{32}^{0.100}X_{30}^{0.024}$$  \hspace{1cm} (Eq. 3)

The parameters in Equation 3 are obtained from Equation 2 as follows

$$h_{11,11} = (\partial V_{-11}/\partial X_{11})(X_{11}/V_{-11}) = 0.317$$
$$h_{11,12} = (\partial V_{-11}/\partial X_{12})(X_{12}/V_{-11}) = -0.00733$$
$$h_{11,16} = (\partial V_{-11}/\partial X_{16})(X_{16}/V_{-11}) = 0.776$$  \hspace{1cm} (Eq. 4)

$$h_{11,32} = (\partial V_{-11}/\partial X_{32})(X_{32}/V_{-11}) = 0.224$$

where the subscript "0" indicates that the results are evaluated at the nominal steady state values.

**DISCUSSION**

Biochemical systems theory provides a step-by-step procedure to formulate the equations that describe any biochemical system. First, the relevant dependent and independent concentration variables are identified and represented by $X$s with appropriate subscripts. For convenience, the dependent concentration variables are numbered consecutively from 1 to $n$, and the independent variables are then numbered from $n + 1$ to $n + m$. Second, the processes that convert one $X$ to another are specified, along with the variables that act as modifiers of these processes. Third, Kirchhoff’s node equations are written for each dependent concentration variable. For the S-system representation in biochemical systems theory, one aggregates influxes and effluxes to produce two aggregate fluxes—one into and the other out of each dependent pool. Finally, the rate law for each aggregate flux is represented by a product of power-law functions. There is one power-law function for each variable that influences the aggregate rate law.

In the case of the tricarboxylic acid cycle for *Dictyostelium*, the variables and processes have been identified in the model proposed by Wright *et al.* (8). The schematic of this model is shown in Fig. 2. The systemic representation of this model in the Michaelis-Menten representation also has been specified by Wright *et al.* (8), and it has been presented here in Equations 1 and A1. The corresponding model in the power-law representation is given in Equations 1 and A2. An example was given to show how one derives the power-law representation from the Michaelis-Menten representation for a single aggregate rate law; all other aggregate rate laws can be derived in the same fashion.

In the following paper of this series (42), we shall evaluate the consistency and robustness of this model of the tricarboxylic acid cycle. It is important to examine these measures of quality for the model so that one can gauge the reliability of predictions that arise from its subsequent analysis (43).

**APPENDIX**

**Michaelis-Menten Representation of the Tricarboxylic Acid Cycle**—The rate laws for the individual reactions that constitute this model were provided by Wright *et al.* (8) and are given below. The parameters have been determined from enzyme kinetic measurements in vitro and from tracer studies in vivo.

$$v_{12} = X_{30}X_{1}$$
$$v_{17} = X_{33}X_{1}$$
$$v_{17} = X_{33}X_{1}$$
$$v_{20} = X_{29}X_{2}X_{1}/(0.00700X_{2} + 0.0100X_{1}(1 + X_{2}/0.110) + X_{2}X_{1})$$
$$v_{410} = X_{20}X_{3}X_{1}/(0.340X_{4} + 0.130X_{1}(1 + X_{2}/0.0210) + X_{4}X_{1})$$
S-system Representation of the Tricarboxylic Acid Cycle—
The aggregate rate laws for synthesis and degradation of each metabolite are derived from the aggregate rate laws in the Michaelis-Menten representation, as shown in Equations 2–4. The results are the following:

\[
V_1 = 0.825X_1^{0.030}X_2^{0.223}X_3^{0.166}X_4^{0.180}X_5^{0.180}X_6^{0.180}X_7^{0.180}X_8^{0.180}X_9^{0.180}
\]

\[
V_2 = 1.34X_1^{0.315}X_2^{0.315}X_3^{0.315}X_4^{0.315}X_5^{0.315}X_6^{0.315}X_7^{0.315}X_8^{0.315}X_9^{0.315}
\]

\[
V_3 = 0.0653X_1^{0.720}X_2^{0.720}X_3^{0.720}X_4^{0.720}X_5^{0.720}X_6^{0.720}X_7^{0.720}X_8^{0.720}X_9^{0.720}
\]

\[
V_4 = 16.2X_1^{0.023}X_2^{0.023}X_3^{0.023}X_4^{0.023}X_5^{0.023}X_6^{0.023}X_7^{0.023}X_8^{0.023}X_9^{0.023}
\]

\[
V_5 = X_1X_2
\]

\[
V_6 = 0.152X_3^{0.018}X_4^{0.018}X_5^{0.018}X_6^{0.018}X_7^{0.018}X_8^{0.018}X_9^{0.018}
\]

\[
V_7 = 1.87X_1^{0.027}X_2^{0.027}X_3^{0.027}X_4^{0.027}X_5^{0.027}X_6^{0.027}X_7^{0.027}X_8^{0.027}X_9^{0.027}
\]

\[
V_8 = 0.0192X_1^{0.037}X_2^{0.037}X_3^{0.037}X_4^{0.037}X_5^{0.037}X_6^{0.037}X_7^{0.037}X_8^{0.037}X_9^{0.037}
\]

\[
V_9 = 2.97X_1^{0.030}X_2^{0.030}X_3^{0.030}X_4^{0.030}X_5^{0.030}X_6^{0.030}X_7^{0.030}X_8^{0.030}X_9^{0.030}
\]

\[
V_{10} = 0.030X_1^{0.015}X_2^{0.015}X_3^{0.015}X_4^{0.015}X_5^{0.015}X_6^{0.015}X_7^{0.015}X_8^{0.015}X_9^{0.015}
\]

\[
V_{11} = 1.50X_1^{0.225}X_2^{0.225}X_3^{0.225}X_4^{0.225}X_5^{0.225}X_6^{0.225}X_7^{0.225}X_8^{0.225}X_9^{0.225}
\]

\[
V_{12} = 1.00X_1^{0.180}X_2^{0.180}X_3^{0.180}X_4^{0.180}X_5^{0.180}X_6^{0.180}X_7^{0.180}X_8^{0.180}X_9^{0.180}
\]

\[
V_{13} = 0.942X_1^{0.017}X_2^{0.017}X_3^{0.017}X_4^{0.017}X_5^{0.017}X_6^{0.017}X_7^{0.017}X_8^{0.017}X_9^{0.017}
\]

(Eq. A2)

**REFERENCES**

Tricarboxylic Acid Cycle in Dictyostelium