KINETICS ON THE MICROBIAL SCALE

by H. A. Deans

To chemical engineers the term kinetics implies the attempt to interpret rates of chemical reactions, quite often on the basis of reasonably comprehensive data on the system of interest. The number of reactions involved is usually small, and the worst that can happen is a mass-transfer limitation to obscure the rate mechanism or something of equivalent complexity.

When we first look at the reaction systems which take place in living organisms, we are immediately assaulted by appalling qualitative differences. The number of reactions is large; intermediates are shared by several different reactions; hardly any reaction goes without the very efficient catalysis of an enzyme; and the molecules involved are mostly very large and apparently unnecessarily complicated. My first impulse was to ignore this internal complexity, and to try to apply pseudo-kinetics to the "overall" process of respiration and substrate consumption.

A little bit of reading in the biochemistry literature was sufficient to convince me that this course would lead nowhere. It soon became evident that the interaction of the many reactions occurring in a living cell is of primary qualitative importance, and that any attempt to ignore the details of the mechanisms would miss the point altogether. We are seeking an understanding of how living cells utilize substrates and oxygen, control their own growth, react to changes in environment, and do all the other things that only living cells can do; our only avenue of attack is through the detailed mechanisms of the biochemical reactions which accomplish all these things.

The present state of mechanistic knowledge is such that we can begin the job of formulating the kinetics problem, if nothing else. I will try to write out the algebra for an important set of reactions and show that the mathematical formulation has some of the properties of the living system. The algebraic description can then be a basis for analyzing sensitivity and control of the system, which is a potentially rich source of inverse information.

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Before proceeding, I might mention a working hypothesis on which many of my arguments are based. The question will arise repeatedly, "Why do living cells choose to accomplish 'simple' results in such complicated ways?" One answer is that N billion years of evolution have presumably optimized the internal structure of the cell as far as mechanisms, rates, catalysts, and control are concerned. All we have to do is discover *how* the cell does it, and then the fascinating part can commence.

Another hypothesis, if it can be called that, I alluded to above. We are not going to gain much insight by looking at individual reactions or even reaction paths. There seems to be a "minimal set" of interrelated reactions which will show the features of living systems. If we work with less than this set, the behavior will literally be "dead" in the sense that the system will not produce the characteristic bahavior we are looking for. The reaction set I will describe later is hopefully, at least, a "minimal set," although this judgement is at best subjective. It is based on geometric as well as mechanistic arguments with the final rationale dependent on the assumption of evolutionary optimization which was just mentioned.

Before getting on with the algebra of the example reaction set, I need to make some remarks about transport limitations and chemical kinetics restrictions.

Time and Distance Scales in Determining Overall Rates

The "global" processes involved when a cell takes up nutrients, etc., take place on a distance scale of the order 10^{-4} cm in a "well-mixed" culture. This overall process necessarily involves diffusive and membrane transport steps in series with the eventual chemical reaction paths. The example I have in mind is the ubiquitous E. Coli, assumed to be metabolizing glucose.

The average E. Coli, during its twenty-minute half-life, takes glucose through its membrane at about 10^{-10} moles/cm^2-sec. If we allow a "rich" driving force of 10^{-7} moles/cm^3 operating over a distance equal to the cell diameter, we arrive at a quasi-diffusion coefficient of order 10^{-7} cm^2/sec. If the actual diffusivity of glucose is larger than this, which it should be, then diffusion does not seriously limit the overall process. Of course, there are systems which are external diffusion limited; I will choose to ignore them.

This takes care of diffusion from the membrane outward. The only argument which we can employ to justify discounting *internal diffusion* as an absolutely controlling rate step is heuristic. Presumably the cell has evolutionary control over internal processes, and will not let itself be limited by factors it can change. There is in fact evidence that the enzymes which catalyze a complete set of reactions are located in close

proximity (10^{-5} or 10^{-6} cm) to one another. Even though very large complicated intermediates are involved, their diffusivities are probably large enough so that they can get from one enzyme to the next without producing serious diffusional limitation.[1]

The question of time scales is important, since I would like to employ the "steady-state" hypothesis in dealing with the reaction set. This is merely the assumption that the rate of accumulation of intermediates can be neglected on the "reaction time scale." We can use E. Coli again to give us an idea of the order of magnitude of this time scale.

E. Coli uses glucose at an average rate of 5×10^{-6} moles/cm^3-sec. If we suddenly shut off the glucose supply from outside, the bacterium would use up its internally stored glucose in approximately one second. This is, then, the "reaction time scale," and it is about a thousand times shorter than the "half-life." We can probably get away with the steady-state hypothesis in an environment where changes occur on the order of minutes or longer, i. e., on the cell-division time scale rather than on the reaction time scale.

In summary, we will focus on a neighborhood of order 10^{-5} cm in diameter in which a large set of reactions is assumed to occur without controlling diffusional rate limitation. A few reactants will pass in, a few products will leave, and a large number of intermediates will exist locally at stationary concentration, at least on the time scale of interest. The only thing to consider then is kinetics.

Elementary Steps and Kinetic Expressions

One of the first things a good student learns in a modern kinetics course is that a knowledge of stoichiometry is necessary but not sufficient. We cannot just look at the equation of an overall reaction and guess the form of the kinetic expression which will describe the overall rate. Especially in the case of heterogeneously catalyzed reactions, we must know or guess the detailed mechanism by which the reaction proceeds. We then write down the series of chemical equations which describe the mechanism, make sure they add up to the overall reaction, and then we try to write kinetic expressions to account for the steps.

This procedure is also appropriate in the case of biochemical reaction schemes. The prospect is frightening at first glance because of the large number of reactions and catalysts involved. However, complicated relations are inevitable, since the behavior we wish to model is extremely complex.

Some of the qualitative features which our kinetic equations must include are 1) the effects of shared intermediates in two or more complex reaction paths; 2) the limitation imposed by closed cycle reaction pathways; 3) the use of a specific enzyme to catalyze each step, the total

amount of enzyme being fixed; 4) the dependence of enzyme catalytic activity on concentrations of "foreign" bodies, i. e., substances not involved in the step catalyzed by the enzyme; and 5) the relative reversibility of most elementary steps, as opposed to the energetic irreversibility of the overall reactions.

I will proceed confidently, assuming that I know or can guess the detailed mechanisms of the reactions which will follow. This implies a knowledge of all the enzymes and their idiosyncracies, which is certainly not true even in the well-studied case I have chosen. What I will do in fact is propose a scheme which is sufficiently complex to reproduce the known behavior of the system at steady state. This involves writing kinetic expressions, imposing mass conservation conditions on enzymes and intermediates, and doing some elementary algebra.

The Example Reaction Set

My example set is the group of reactions by which many organisms regenerate the indispensible and ubiquitous phosphate compound ATP. The source of biochemical energy is the breakdown of a substrate, e. g., glucose, to CO_2 and H_2O, a process which involves many enzyme catalyzed steps. I am not going to worry about how the cell reduces glucose to the three-carbon residues which are used by the extended Krebs cycle; this was discussed in an earlier paper, and is not difficult to separate in principle (and in fact) from the reaction system I will be concerned with.

The example reaction set is found in organisms ranging from E. Coli to man. Geometrically, the various steps take place in a subregion of a particular cell which is quite small. From here on, we are concerned only with this microcosm, which takes in oxygen, phosphate, ADP, and pyruvic acid (the three-carbon fuel), and returns CO_2, H_2O, and ATP to the surroundings.

There are two major reaction pathways involved in the set. These are 1) the expanded tricarboxylic acid (Krebs) cycle, which converts pyruvate to CO_2 and produces a number of equivalents of reduced coenzyme intermediates, and 2) the electron transport path, which reoxidizes the coenzymes so that they can be used again in the first cycle, and in the process converts phosphate and ADP to ATP. A detailed description of these reaction paths can be found in any modern biochemistry text.

I have attempted to abstract the essential features of these systems in Figures 1 and 2. I have let I_i be a typical intermediate in the Krebs cycle, with I_0 being pyruvate. O_i and r_i are oxidized and reduced forms of intermediate i in the electron transport pathway. In both cases, the equations shown are the overall stoichiometric ones, and do not include the details of the actual fundamental steps. ATP formation is apparently

O_i = OXIDIZED FORM OF REDOX INTERMEDIATE i $i = 1, \ldots N$

R_i = REDUCED FORM OF REDOX INTERMEDIATE i $N \stackrel{?}{=} 7$

$$(1) \qquad O_1 + R_2 \rightleftharpoons O_2 + R_1$$

$$(i) \qquad (ADP + P) + O_i + R_{i+1} \rightleftharpoons O_{i+1} + R_i + (ATP + H_2O)$$

$$(N-1) \qquad ADP + P + O_{N-1} + R_N \rightleftharpoons O_N + R_{N-1} + ATP + H_2O$$

$$\text{OVERALL:} \quad 3ADP + 3P + O_1 + R_N \longrightarrow O_N + R_1 + 3ATP + 3H_2O$$

FIG. 1 - ELECTRON TRANSPORT PATHWAY STOICHIOMETRY

connected with the details of catalyst regeneration in the electron transport scheme, and thus, does not show up explicitly in the stoichiometry for the redox steps.

I_i = Intermediate i $i = 0, 1, \ldots M$ $M \stackrel{?}{=} 9$

$$(1) \qquad I_0 + O_N \rightleftharpoons I_1 + R_N + CO_2$$

$$(2) \qquad I_1 + I_M \rightleftharpoons I_2$$

$$(j) \qquad (H_2O) + [O_N] + I_{j-1} \rightleftharpoons I_j + [R_N] + \{CO_2\}$$

$$(M) \qquad I_{M-1} + O_N \rightleftharpoons I_M + R_N$$

$$\text{OVERALL:} \quad 3H_2O + I_0 + 5O_N \longrightarrow 5R_N + 3CO_2$$

FIG. 2 - KREBS CYCLE STOICHIOMETRY

I have simplified the coupling between the two pathways slightly. Only the O_N-R_N pair appears in the Krebs cycle, although strictly speaking O_{N-1} – R_{N-1} should be involved in one step. In fact this oversight is compensated stoichiometrically by another step outside the extended Krebs cycle, which produces the pyruvate from malate. The effect on the overall scheme should be small.

The notation I have used may appear unnecessarily general. However,

there are about ten of the I_i and at least seven each of the O_i and R_i, all with formidable organic chemical names. In the case of the electron transport scheme, there is enough variation among species to justify the flexibility. Finally, we want to be able to recover when somebody discovers a new step in the chain, which is not unlikely.

We are not in a position to derive kinetic relations until we write down the fundamental steps of the individual stoichiometric equations. This information is not known definitely for any of the reactions involved, and can only be guessed at for some. The following is an attempt at such guesswork; Figure 3 shows a possible mechanism for one of the electron

E_i = THE SPECIFIC ENZYME FOR STEP i

A_j = ANY CHEMICAL SPECIES PRESENT

(1) $E_i + A_j \rightleftharpoons (\overset{A}{E})_{ij}$ (MODIFIED CATALYST)

(2) $O_i + (\overset{A}{E})_{ij} \rightleftharpoons (O\overset{A}{E})_{iji}$ OR $R_{i+1} + (\overset{A}{E})_{ij} \rightleftharpoons (\overset{A}{E})_{iji+1}$

(3) $R_{i+1} + (O\overset{A}{E})_{iji} \rightleftharpoons (\overset{A}{E}O)_{iji+1} + R$ OR $O_i + (\overset{A}{E}R)_{iji+1} \rightleftharpoons (R\overset{A}{E})_{iji} + O_{i+1}$

(4) $(\overset{A}{E}O)_{iji+1} \rightleftharpoons (\overset{A}{E})_{ij} + O_{i+1}$ OR $(R\overset{A}{E})_{iji} \rightleftharpoons (\overset{A}{E})_{ij} + R_i$

NET: $O_i + R_{i+1} \rightleftharpoons O_{i+1} + R_i$ $\quad O_i + R_{i+1} \rightleftharpoons O_{i+1} + R_i$

FIG. 3 - HYPOTHETICAL DETAILED MECHANISM OF ONE STEP IN THE ELECTRON TRANSPORT PATHWAY

transport steps. Briefly, (1) states that the enzyme for step i can "react," (adsorb on its large and complex surface) various species A_j; this is the allosteric mechanism, and generalizes the function of a catalyst significantly. The modified form $\overset{A}{E}_{ij}$ may be either more or less active as a catalyst than E_i, so that the concentration of A_j may retard or enhance the rate of the reaction although it has nothing to do with the stoichiometry; (2) through (4) are the possible steps for the accomplishment of the overall purpose. A similar mechanism can be implied for any one of the steps in the Krebs cycle.

An analogous mechanism for the electron transport steps which include ATP formation is shown in Figure 4. More complicated schemes can easily be constructed. Any mechanism which is less complicated, in that it allows the redox step without ATP formation, would have to be rejected. Oxygen utilization apparently does not proceed without phosphate fixation.

The Kinetics of the Example Set

It might be well to state an objective before proceeding with the

Z = "ACTIVATED" COUPLING ENZYME

(1) $R_{i+1} + Z \rightleftharpoons (ZR)_{i+1}$

(2) $O_i + (ZR)_{i+1} \rightleftharpoons \{ZO\}_{i+1} + R_i$

(3) $\{ZO\}_{i+1} + P \rightleftharpoons \{ZP\} + O_{i+1}$

(4) $\{ZP\} + ADP \rightleftharpoons Z + ATP + H_2O$

FIG. 4- A POSSIBLE MECHANISM FOR PHOSPHATE FIXATION IN THE ELECTRON TRANSPORT PATHWAY

algebra of the kinetic relations. Clearly we are going to end up with a large number of parameters "to be determined" in the expressions we will derive, simply because of the large number of reactions and elementary steps. The usual objective of "fitting the data to the theory" and evaluating the constants is obviously out of the question. The best we hope for is a set of equations which we can subject to certain tests. These will necessarily be of the plausibility type, i. e., can we find a set of physically meaningful parameters such that the mathematical system is a reasonable model of the living system.

In Figure 5 I outline the procedure for obtaining the rate equation for

ALL SYMBOLS NOW REPRESENT CONCENTRATIONS

(1) $\dfrac{(^{A}_{E})_{ij}}{(E_i)(A_j)} = K_{ij}$ (THE K_{ij} ARE EQUILIBRIUM CONSTANTS)

$(r_1)_i = \sum_j \{ f^1_{ij} O_i (^{A}_{E})_{ij} - b^1_{ij} (O^{A}_{E})_{iji} \}$

(2) $(r_2)_i = \sum_j \{ f^2_{ij} (O^{A}_{E})_{iji} R_{i+1} - b^2_{ij} R_i (^{A}_{E}O)_{iji+1} \}$

$(r_3)_i = \sum_j \{ f^3_{ij} (^{A}_{E}O)_{iji+1} - b^3_{ij} (^{A}_{E})_{ij} O_{i+1} \}$

THE f^k_{ij} ARE FORWARD RATE CONSTANTS, THE b^k_{ij} ARE REVERSE RATE CONSTANTS

AT STEADY STATE: $(r_1)_i = (r_2)_i = (r_3)_i$

CONSERVATION OF ENZYME i : $(E_i) + \sum_j \{ (^{A}_{E})_{ij} + \cdots\cdots + (^{A}_{E}O)_{iji+1} \} = E^o_i$, (CONSTANT)

(3) $r = f^*_i (O_i)(R_{i+1}) - b^*_i (O_{i+1})(R_i)$

WHERE f^*_i, b^*_i ARE FUNCTIONS OF $(A_j), (O_i), R_i, O_{i+1}, R_{i+1}$ AND THE CONSTANTS $k_{ij}, f^k_{ij}, b^k_{ij}, E^o_i$

FIG. 5- RATE EQUATIONS AND ALGEBRA FOR TYPICAL STEP IN A PATHWAY

a single "overall" reaction. First we assume the catalyst modification steps are at equilibrium. Then we write reversible rate equations for each primary step; we employ the steady state hypothesis along with the conservation of enzyme i, to eliminate the intermediate unknowns; and finally, we write the resulting rate in terms of reactant-product concentrations and the pseudo-constants.

For the redox reaction in which ATP is produced, a similar process will yield f_i^* and b_i^* which depend upon the concentrations of ADP, phosphate, and ATP. The functionality will be such that the rate will approach zero if either phosphate or ADP concentration vanishes.

Beginning in Figure 6 I have faced the problem of determinancy in

ASSUME (O_1), OXYGEN, AND (R_1), WATER ARE FIXED INPUT PARAMETERS

EQUATIONS		UNKNOWN INTERMEDIATES, ETC.	
$r = f_i^*(O_i)(R_{i+1}) - b_i^*(O_{i+1})(R_i)$	6	O_i $i = 2, \ldots 7$	6
$O_i + R_i = T_i$ $i = 2 \ldots 7$	6	R_i $i = 2, \ldots 7$	6
	r		
	12		13

NOTE: IN THE STEADY STATE, $r = 5\bar{r}$, WHERE $\bar{r}$ IS THE RATE OF USE OF I_0 IN KREBS CYCLE

FIG. 6 - DETERMINACY IN THE ELECTRON TRANSPORT PATHWAY

the reaction set. The facts to recall when counting unknowns and equations are that 1) the total amount of any $O_i - R_i$ pair, namely T_i, is fixed; 2) the f_i^* and b_i^* contain only the O_i and R_i and constants, i. e., the unknown catalyst concentrations have been eliminated; a similar process yields a similar situation for the f_i and b_i in the Krebs cycle which we see in Figure 7; 3) the overall rate of the electron transport pathway is five times the rate of the Krebs cycle "rotation," because of stoichiometry; and 4) O_N and R_N are unknowns in *both* the Krebs and redox systems.

We see that there is an excess in unknowns of 1. That is, if we set I_0 (fuel), O_1 (oxygen), and R_1 (water) compositions, the system is still underdetermined by one. The difficulty stems from the cyclic structure of the Krebs system, and is characteristic of cyclic stoichiometric schemes. One of the intermediates (in the simplest case) $I_2 - I_9$ must be set in the steady state by an additional restriction. I have shown this simplest case in Figure 8, with I_8 (Malic acid) picked as the base intermediate for no good reason.

ASSUME (I_0), PYRUVATE, IS A FIXED INPUT PARAMETER

EQUATIONS		UNKNOWN INTERMEDIATES, ETC.	
$\bar{r} = 5r$	1	I_i	9
$\bar{r} = f_1(I_0)(O_N) - b_1(I_0)(R_N)$		$\bar{r}$	1
$\bar{r} = f_2(I_1)(I_9) - b_2(I_2)$			
⋮			
$\bar{r} = f_9(I_8)(O_N) - b_8(I_9)(R_N)$	9		10
FROM ELECTRON TRANSP.	12		13
GRAND TOTAL	22 EQ'NS		23 UNKNOWNS

FIG. 7 - DETERMINACY IN THE KREBS CYCLE AND IN IN THE ENTIRE REACTION SYSTEM

Presumably there is an enzyme factory which can synthesize I_8 at arbitrarily small rate r_s. I_8 is also lost by diffusion from the system at rate MI_8, where M is a small mass transfer coefficient. The steady state value for I_8, r_s/M, is the ratio of two small numbers, and can presumably be whatever the cell desires. The important fact is that the overall rate

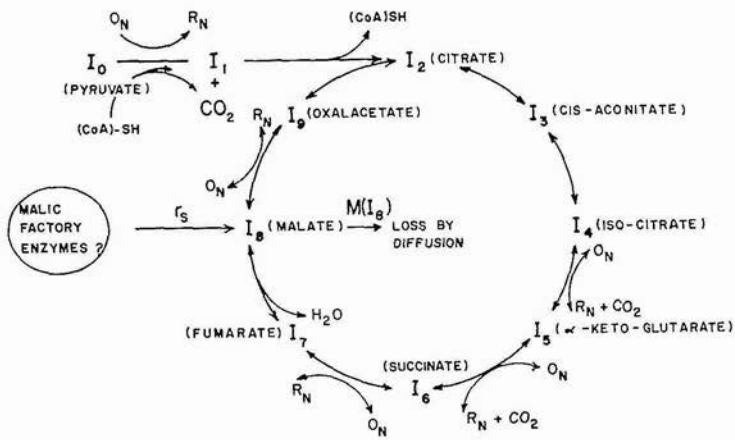

FIG. 8 - MODIFIED KREBS CYCLE (SIMPLIFIED)

r is directly proportional to r_s/M in the steady state.

Since normally M would be fixed by cell morphology, the overall rate of ATP formation can be controlled by the cell merely by changing the small rate r_s. This is the principle of the power amplifier and is characteristic of cyclic reaction paths. It will be the subject of a demonstration in the next paper.

Summary

The reaction system which I have described mathematically has the following features: 1) It is determined when fuel, oxidant, phosphate, and ADP concentrations are given, i. e., the rates and all intermediate concentrations are set; 2) By proper choice of constants the system rate can be made insensitive to oxygen and substrate concentrations, and sensitive to phosphate and/or ADP concentrations; 3) The transient response of the system to changes in external conditions can be made rapid (on the order of the reaction time scale mentioned earlier); and 4) Changes in the level of activity can be governed by alterations in the source-loss ratio (r_s/M), with characteristic response time much longer than the reaction time scale.

Some of the details of the qualitative behavior of the system will be discussed in the next paper. Before I turn my system over to the analog computer, I might interject a skeptical word about using this model to fit real system data. In writing a general kinetic scheme for the Krebs cycle and the Electron Transport scheme, I have necessarily introduced a large number of constants. It might be tempting to try to use this array of adjustable parameters to force a fit with a particular set of data for a bacterial population. Unless the data represented *individual* cell metabolism rates using a single substrate, this would clearly be misuse of the model.

NOTES

1. If diffusion is only a contributing rather than a limiting factor, its effect can be absorbed in the kinetics to be presented.
2. This also explains how the cycle can get started in the first place.