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STUDIES ON THE DIFFERENTIAL EQUATIONS OF ENZYME KINETICS. I. BIMOLECULAR SCHEME: SIMPLIFIED MODEL.

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ABSTRACT. The main properties of the solution of the differential system of the model are obtained by qualitative integration. The integral curve is compared with the solutions given by the two classical approximations to the problem: it is shown that the steady-state approximation is to be preferred to the rapid equilibrium theory as a general method and the conditions under which they will furnish accurate results are discussed.

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1. INTRODUCTION.

From the expression of a gene to the storage of metabolic products, from the respiration of a cell to the secretion of antibiotics, nearly all life processes are controlled by enzymatic reactions, that is to say by chemical reactions catalyzed by specific proteins called enzymes whose essential function is often to initiate a reaction which, though thermodynamically possible, never occurs spontaneously: for example, glucose left to itself does not ferment, but in the presence of a minute amount of yeast or yeast extract, ethyl alcohol will be produced.

Thus the kinetic equations of enzymatic processes are of fundamental importance, both for theoretical and practical reasons, but little is known about their solution.

Let us consider the case of a bimolecular mechanism in which an enzyme E reacts reversibly with a substrate S; this results in the formation of an activated complex ES^* which can split reversibly into the enzyme E and the products of the reaction which are globally denoted by P. In standard notations:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES^* \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P \quad ,$$

where the k's are velocity constants. Let e, s, x and p be the concentration of the free (= unbound) enzyme, free substrate, activated complex and products of the reaction respectively; then the differential equations of the kinetic system take the form [1]

$$ds/dt = k_{-1}x - k_1es$$

$$dx/dt = k_1es + k_{-2}ep - (k_{-1} + k_2)x$$

where t is the time, with the initial conditions: $s(0) = s_o$, $x(0) = p(0) = 0$.

Furthermore the equations of conservation read

$$e_o = e + x$$

$$s_o = s + x + p \tag{1.1}$$

There is no known close-form solution of this system, except in the case $k_{-2} = k_1$ [2] and very early enzymologists looked for kinetic models easier to handle.

When $k_{-2} = 0$, that is to say when the second process of the bimolecular scheme is irreversible, one obtains, with the same initial conditions and the same equations of conservation, the system

$$\begin{aligned} ds/dt &= k_{-1}x - k_1es \\ dx/dt &= k_1es - (k_{-1} + k_2)x \end{aligned} \tag{1.2}$$

This is a simplified model as there is now one parameter less to consider and the overall velocity of the reaction, defined as the rate dp/dt of appearance of the products, takes the particularly simple form

$$dp/dt = -(ds/dt + dx/dt) = k_2x \tag{1.3}$$

Again there is no known general solution of this non-linear system but through the introduction of hypotheses endowed with a physical meaning one can get a manageable expression of the velocity: i) in a procedure due to Michaelis and Menten and known as the equilibrium or rapid equilibrium theory, one assumes that the reaction $E + S \rightleftharpoons ES^*$ always proceeds at equilibrium (and then $ds/dt = 0$); ii) in the steady-state approximation due to Haldane and Briggs one assumes that the quantity of activated complex is constant (and then $dx/dt = 0$). In both cases the velocity is found to be a simple function of s. As a matter of fact this is a general phenomenon and

the rapid equilibrium and/or steady-state approximations allow one to obtain dp/dt as an algebraic function of the parameters of the differential system even when the reaction scheme is more complex than the simplified bimolecular process, for instance when one considers the effect of inhibitors. Hence within the framework of these approximations the problem is reduced to the evaluation of certain parameters or groups of parameters from a comparison of the theoretical expression of the velocity with its experimental value in specified conditions. But the early methods proposed for the exploitation of such data are rather unreliable [1] and the accurate and systematic study of (simplified) kinetic models could not be carried out before the development of structure analysis [3,1]. In this method, specifically designed to handle small size samples, one does not only calculate efficient and unbiased estimates of the parameters of a given mathematical structure (here the expression of the velocity as a function of the various parameters of the differential system) but one also computes a statistic which measures the overall degree of concordance between that mathematical structure and the set of experimental data, taking into account the physical nature of the experimental technique(s) used. It turns out in particular that the degree of concordance obtained with hypothetical data in which one introduces known errors is more or less what one would intuitively expect while this is not always the case with experimental data which, as far as one can judge, are reliable and the goodness of fit may be too low. This of course suggests that the mathematical expression may not be quite correct and in turn calls for an examination of the mathematical meaning of the two hypotheses $ds/dt = 0$ and $dx/dt = 0$.

Thus the purpose of this paper is to describe how the rapid equilibrium and steady-state approximations are related to the manifold of solutions of the differential system (1.2). In addition the stability of this system will be investigated: this is a problem of importance because chains of enzymatic reactions are frequently encountered in nature.

2. A QUALITATIVE STUDY OF SYSTEM (1.2).

Through substitution of the equation of conservation (1.1) into system (1.2), the latter becomes

$$\begin{aligned} ds/dt &= -k_1 e_o s + k_{-1} x + k_1 s x \\ dx/dt &= k_1 e_o s - (k_{-1} + k_2) x - k_1 s x \end{aligned} \tag{2.1}$$

with the initial conditions

$$s(0) = s_o \quad \text{and} \quad x(0) = 0 \quad . \tag{2.2}$$

The solution of physical interest is such that $s, x \geq 0$.

THEOREM 2.1. The positive solution of problem (2.1)-(2.2) has the following properties:

i) $s(t)$ is monotonically decreasing from s_o to 0 as t increases from 0 to ∞;

ii) there exists a time T such that $x(t)$ increases for $t < T$ and decreases for $t > T$, with $\lim_{t\to\infty} x(t) = 0$.

PROOF. In the phase plane (s,x) system (2.1) satisfies

$$dx/ds = [k_1e_os - (k_{-1} + k_2)x - k_1sx]/(-k_1e_os + k_{-1}x + k_1sx) \tag{2.3}$$

and this equation is considered in the first quadrant. From

$$k_1e_0s - (k_{-1} + k_2)x - k_1sx = 0 \tag{2.4}$$

one obtains the equation of the isocline of zero i_o as

$$x = k_1e_0s/(k_1x + k_{-1} + k_2) \tag{2.5}$$

and from

$$-k_1e_os + k_{-1}x + k_1sx = 0 \tag{2.6}$$

one obtains the equation of the isocline of infinity i_∞ as

$$x = k_1e_os/(k_1s + k_{-1}) \quad . \tag{2.7}$$

By adding equations (2.4) and (2.6) one obtains $x = 0$, and the substitution of the latter value into (2.4) yields $s = 0$. This shows that $s = 0$, $x = 0$ is the only singular point of equation (2.3). Geometrically, it means that (0,0) is the only point of intersection of the curves (2.5) and (2.7). Now, in the first quadrant the isocline of zero (2.5) lies below the isocline of infinity (2.7) since the parameters e_o and k's are positive. Furthermore, as s increases, the graphs (2.5) and (2.7) increase monotonically and approach asymptotically the horizontal line $x = e_o$. Thus (see Fig.1) the curves (2.5) and (2.7) split the first quadrant into three different regions:

R_1: $\quad 0 \leqq x < k_1e_os/(k_1s + k_{-1} + k_2)$,

R_2: $\quad k_1e_os/(k_1s + k_{-1} + k_2) \leq x < k_1e_os/(k_1s + k_{-1})$,

R_3: $\quad k_1e_os/(k_1s + k_{-1}) \leq x < \infty$.

In R_1 we have

$$k_1e_os - (k_{-1} + k_2)x - k_1sx > 0 \quad ,$$

$$-k_1e_os + k_{-1}x + k_1sx < 0 \quad .$$

Hence $ds/dt < 0$ and $dx/dt > 0$. Therefore $s = s(t)$ decreases and $x = x(t)$ increases, while in R_2 we have

$$k_1e_os - (k_{-1} + k_2)x - k_1sx < 0 \quad ,$$

$$-k_1e_os + k_{-1}x + k_1sx < 0 \quad ,$$

which implies $ds/dt < 0$ and $dx/dt < 0$. Hence both $s(t)$ and $x(t)$ decrease in R_2. Fi-

nally, we have in R_3

$$k_1 e_o s - (k_{-1} + k_2)x - k_1 sx < 0 \quad ,$$

$$-k_1 e_o s + k_{-1} x + k_1 sx > 0 \quad ,$$

that is $ds/dt > 0$ and $dx/dt < 0$, which shows that $s(t)$ is increasing in R_3 and $x(t)$ is decreasing.

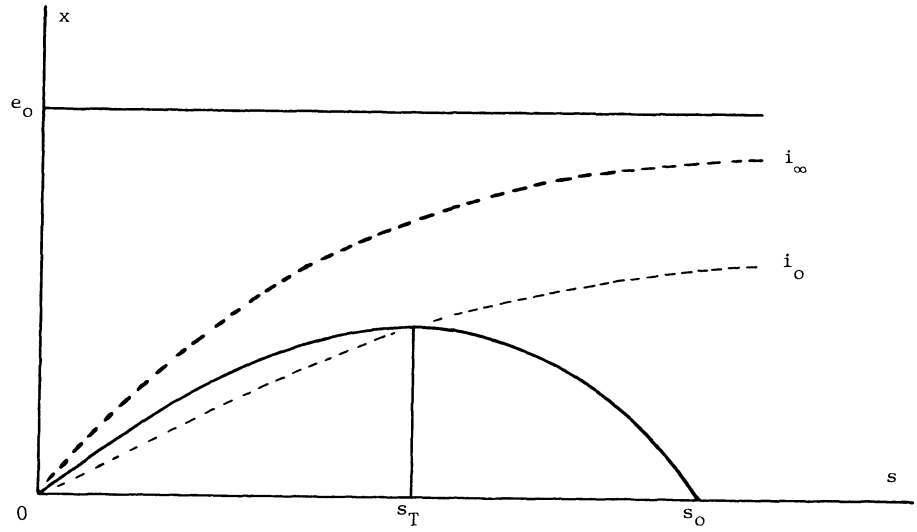

Fig.1

Let us consider that trajectory of the system (2.1) which starts from the point $(s_o,0)$ at $t = 0$. For $t > 0$ this curve first moves upwards to the left in the region R_1. However it cannot remain in R_1 for all $t > 0$ as then $x(t)$ would attain a maximum and decrease in R_1, which is impossible. Therefore at some time T the trajectory must cross the isocline of zero (2.5) and enter the region R_2. In fact it remains in R_2 for all $t > T$, progressing to the left and downwards: it cannot cross back into R_1 since $x(t)$ increases there and it cannot enter R_3 either, because it would have to cross the isocline of infinity (2.7) in the direction of increasing values of s, which is impossible. We conclude that $s(t)$ monotonically decreases for all $t > T$ and that $s(t) \to 0$ as $t \to \infty$. Furthermore $x(t)$ increases for $t < T$ and decreases for $t > T$, with $x(t) \to 0$ as $t \to \infty$. Hence the zero solution of (2.1) is asymptotically stable.QED

The function $x(t)$ is null at the starting point and positive elsewhere in the first quadrant. Then it follows from (1.3) that the overall velocity of the reaction increases monotonically and that $s(t) + x(t)$ decreases. Introducing the function

$$V(s,x) = s + x \quad ,$$

we see that $V(0,0) = 0$ and $V(s,x) > 0$ elsewhere in the first quadrant. Moreover

$$dV[s(t),x(t)]/dt = d(s + x)/dt = -k_2x < 0$$

for $x > 0$. Hence $V(s,x)$ can be considered as a Liapunov function to prove that the solution of the problem (2.1)-(2.2) tends to 0 as $t \to \infty$.

THEOREM 2.2 The integral curves of (2.1) can be divided in the phase plane in two sets: the curves of one class enter the origin with the slope m_1, and the curves of the other with the slope m_2, where m_1 and m_2 are the solutions of the equation

$$k_{-1}m^2 + (k_{-1} + k_2 - k_1e_o)m - k_1e_o = 0 \quad , \tag{2.8}$$

PROOF. Introducing polar coordinates

$$s = r\cos\theta \qquad x = r\sin\theta$$

and observing that

$$r^{-2}d\theta/dt = sdx/dt - xds/dt \quad ,$$

we obtain

$$r^{-2}d\theta/dt = H(s,x) + M(s,x)$$

with

$$H(s,x) = k_1e_os^2 - (k_{-1} + k_2 - k_1e_o)sx - k_{-1}x^2$$

and

$$M(s,x) = -k_1sx(s + x) \quad .$$

As $t \to \infty$, $H(s,x) \to 0$ as r^2 and $M(s,x) \to 0$ as r^3. Therefore the behavior of θ as $t \to \infty$ is determined by $H(s,x)$. The slope m of an integral curve as $t \to \infty$ is the limiting value of x/s. Hence it satisfies $H = 0$ and the latter equation can be written in the form (2.8). It is easily verified that it has two real roots. The origin is a nodal critical point for the linear system corresponding to (2.1).

3. RAPID EQUILIBRIUM THEORY AND STEADY-STATE APPROXIMATION.

The preceding results furnish in particular a basis for the comparison of the rapid equilibrium theory and the steady-state approximation regarding the accuracy with which they can represent the enzymatic process. Moreover they shed some light on the nature of these two models of a bimolecular reaction: the fundamental assumption of the rapid equilibrium theory ($ds/dt = 0$) leads to the equation of the isocline of infinity (2.7) while the fundamental assumption of the steady-state approximation ($dx/dt = 0$) leads to the equation of the isocline of zero (2.5). Now when the reaction has proceeded for a certain time $> T$ the trajectory lies between these two isoclines, so that asymptotically - i.e. near the end of the reaction - there is little reason to select one model over the other.

A region of greater interest from the point of view adopted here is that domain of the phase plane corresponding to the beginning of the reaction. While the substrate concentration decreases from s_o to nearly s_T (see Fig.1) the integral curve remains below the isocline of zero, and the latter lies below the isocline of infinity. Thus in this region the steady-state approximation (which corresponds to the isocline of zero) constitutes a better model than the rapid equilibrium theory (which corresponds to the isocline of infinity).

Now the maximum discrepancies between these curves occur at $s = s_o$. Thus the deviations Δx at $t = 0$ between the integral curve and each of the isoclines considered is a measure of the error due to each approximation. In the case of the rapid equilibrium theory this deviation is

$$\Delta x_{RE} = k_1 e_o s_o/(k_1 s_o + k_{-1}) \tag{3.1}$$

and in the case of the steady-state approximation

$$\Delta x_{SS} = k_1 e_o s_o/(k_1 s_o + k_{-1} + k_2) \quad . \tag{3.2}$$

Introducing the quantity $K_M = (k_{-1} + k_2)/k_1$, a parameter known as the Michaelis constant of the reaction, one can put equation (3.2) in the form

$$\begin{aligned} \Delta x_{SS} &= e_o s_o/(s_o + K_M) \\ &= e_o[1 - K_M/(s_o + K_M)] \quad . \end{aligned} \tag{3.3}$$

The proportionality of Δx_{SS} to e_o is not surprising: x cannot at any time be greater than e_o and therefore, if e_o is very small, dx/dt is nearly zero and the basic assumption of the steady-state approximation is fulfilled. Somewhat unexpected, on the other hand, is the result concerning the influence of the initial concentration of substrate s_o. When assaying an enzyme in vitro, one tends to shy away from using very dilute solutions of substrate because of the experimental difficulties often associated with such preparations (e.g. the correction for spontaneous hydrolysis may become important) and in consequence, one naturally tends to attribute less weight to data obtained under these conditions. It follows, however, from formula (3.3) that all other things being equal, results obtained with very low concentrations of substrate and analyzed following the Briggs-Haldane scheme are bound to be more correct than those obtained with relatively high concentrations.

Similarly, one can cast the error term associated with the rapid equilibrium theory (3.1) in the form

$$\Delta x_{RE} = e_o[1 - K/(s_o + K)]$$

where $K = k_{-1}/k_1$ is the dissociation constant of the activated complex. As in the

case of the steady-state approximation, the error decreases with e_o and s_o; however this time it is the effect of s_o which was predictable (as a very small value of s_o justifies the assumption $ds/dt = 0$) and the effect of e_o which is unexpected.

The importance of the term $\Delta x_{RE} - \Delta x_{SS}$ remains to be examined when e_o and s_o are given: this difference will be negligible is the quantity $K_M - K$ is negligible, which requires in turn that k_{-1} be much greater than k_2. The latter condition means that the dissociation of the activated complex into the products of the reaction should be slow enough not to perturb appreciably the equilibrium between the free enzyme and the activated complex. This is a rash assumption [1] and therefore its realization is of uncertain occurrence, and, except when e_o and/or s_o are very small, the quantity $\Delta x_{RE} - \Delta x_{SS}$ is likely to be significantly different from zero for most enzymatic reactions. Thus, loosely speaking, one should often expect in practice to find the isoclines of zero and infinity appreciably separated near $t = 0$.

This is to say that the case for the rapid equilibrium theory is rather weak and that the steady-state approximation is to be preferred as a general method.

Finally it must be pointed out that the form of the error term Δx_{SS} (and that of Δx_{RE} as well) suggests that when several sets of experimental data referring to the same enzymatic reaction are available, the analysis of the results will be facilitated by carrying out an extrapolation to zero initial concentration of the enzyme and/or the substrate.

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