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A Stochastic Approach to Statistical Kinetics with Application to Enzyme Kinetics*

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A new mathematical approach to chemical kinetics which takes into account inherently random aspects of mechanisms of action is applied to the Michaelis-Menten hypothesis. The stochastic difference-differential equation which defines a *stochastic model* for the basic enzyme-substrate reaction is obtained. The probability parameters of the process, which correspond to the ordinary rate constants of the classical equations, are introduced both *axiomatically*, following the general mathematical theory, and by means of *collision theory*. The stochastic Markovian equation gives the rate of change of the probability function of the concentrations of substrate and enzyme-substrate complex; it parallels the system of ordinary differential equations of the classical mathematical model. The classical and stochastic theories are compared in terms of these equations: it is shown that the *deterministic* differential equations may be obtained from the stochastic model by a process of averaging. Thus, the new approach provides a more comprehensive mathematical framework. The stochastic theory predicts randomness, whereas the deterministic theory must add experimental error terms to the basic equations in order to accomodate the random irregularities actually observed in kinetic data. Accordingly, two different types of *irreproducibility* must be distinguished: *experimental* and *inherent*. The latter is characterized by the stochastic model presented in this paper, which brings kinetics into closer relation with the statistical (thermodynamic) treatments of the equilibrium state.

1. INTRODUCTION

During the past 10 years[1] a variety of chemical reaction mechanisms have been examined from the probabilistic point of view of the theory of stochastic processes. The emergent "stochastic models" for various reaction types suggest a general "statistical kinetics" (Bartholomay, 1957). In this paper the new approach will be related to enzyme kinetics.

2. GENERAL AIMS OF THE STOCHASTIC APPROACH TO CHEMICAL KINETICS

The change in approach which the statistical, or stochastic, treatment of kinetics represents relative to the classical deterministic kinetics may be likened to the relationship of statistical mechanics to classical physical mechanics. Whereas classical kinetics has encouraged the conception of the kinetics of a reaction process as the transformation of chemical substances *in bulk*, concentrations forming the units of continuous transformation, the statistical approach grows out of the explicit treatment of these same transformations as compositions of individual molecules and discrete inter- and intramolecular events. The large numbers of such individual molecular events are of course subsumed in the classical treatment and find a place in the "rate constants," but the emergent rate equations have only a *deterministic* context—which excludes the possibility of random fluctuations and cannot, therefore, be directly decomposed into the contributing random events. On the other hand, starting with the more fundamental principles of stochastic models one may pass by averaging processes to the traditional treatment, which therefore finds a place in the new theory in the sense of a central tendency, around which statistical fluctua-

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[1] See bibliography at end of chapter 8 in Bharucha-Reid (1960).

tions are explicitly permitted and their quantities determined.

Statistical analyses of kinetic data based on the usual deterministic kinetics can be justified only on the assumption that the smooth time courses prescribed by analytic (*i.e.*, nonstochastic) concentration-time functions have, in practice, added to them a random variable resulting from the Gaussian laws of errors generally assumed to be operant in all experimental determinations. It is only in this empirical manner that a given kinetic time course can be considered as a statistical entity on which the usual regression methods may be tried. But this in itself reflects an inhomogeneity in approach. Whereas certain considerations of probability are made in deriving the dependency of rate constants on physical parameters and environmental factors, the results are incorporated into the same deterministic rate equation which can be derived directly from the law of mass action (Bartholomay, 1960a).

Thus, the deterministic method implies that if all conditions are held constant, smooth curves are predicted. On the other hand, the appearance of fluctuations in most kinetic data requires a statistical explanation. To account for this, experimental variation has usually been invoked. The stochastic approach questions the absolute validity of this procedure, since it is able to show that, starting with the same statistical principles which are invoked to explain the vagaries of rate constants, random fluctuation is the rule. Independent experimental variations are assumed to be imposed on the intrinsic fluctuations predicted in the stochastic models. Hence, according to the stochastic approach, even in the total absence of experimental irregularities a concentration-time course has an independent existence as a statistical entity.

It must be allowed that in many cases of simple reaction mechanisms the amount of inherent variability would be small and unresponsive to techniques and measuring apparatus that lack the necessary resolving power. On the other hand, the existence of macroscopic fluctuations (in certain so-called "irreproducible" reactions) has been documented by experiment (Singer, 1953). The sources of randomness in dealing with discrete molecular events may be found in the Brownian-like motions of the reactant molecules, in the random intermolecular collisions, and in the accompanying intramolecular "random walks" from one discrete quantum energy level to another.

Before we proceed with the stochastic treatment, a brief resumé will be given of the general problem of classical deterministic kinetics.

3. Classical Deterministic Kinetics

Let a very general reaction mechanism be describable stoichiometrically by the following system of chemical equations[2]:

$$\left\{ {}^{(i)}a_1{}^{(i)}A_1 + {}^{(i)}a_2{}^{(i)}A_2 + \cdots + {}^{(i)}a_{ji}{}^{(i)}A_{ji} + \cdots + {}^{(i)}a_{ri}{}^{(i)}A_{ri} \underset{k_{-i}}{\overset{k_i}{\rightleftharpoons}} {}^{(i)}b_1{}^{(i)}B_1 + {}^{(i)}b_2{}^{(i)}B_2 + \cdots + {}^{(i)}b_{ki}{}^{(i)}B_{ki} + \ldots + {}^{(i)}b_{si}{}^{(i)}B_{si} \right\} \quad (1)$$

where $(i = 1,2,\ldots,N)$ and where the subscripts on the rate constants k and the superscripts to the left of the integral a- and b- coefficients and of the chemical A- and B- species identify the ith (reversible) reaction type.

By the *deterministic model* for the kinetics of this reaction is understood a system of differential equations, at most $\sum_{i=1}^{N}(r_i + s_i)$ in number, one corresponding to each of the product and reactant-species. In each equation the time derivative of one of these concentrations appears on the left, and a function of this and, possibly, of all other concentration-variables and of the parameters k and of time, t, on the right. Since this system of equations is not necessarily an independent system, owing to interrelationships between the variables, it may be expressed usually in terms of a fewer number, say R, of these equations. For convenience, then, referring to the resulting "canonical" reactant- and product-concentration variables simply as $A_1, A_2, \ldots, A_r, \ldots, A_R$, the deterministic model may be represented in canonical form as:

$$\frac{dA_r}{dt} = f_r(A_1,A_2,\ldots,A_r,\ldots,A_R;\ k;\ t) \quad (r = 1,2,\ldots,R) \quad (2)$$

(together with appropriate initial and "material balance" conditions [Bartholomay, 1960a]) where k represents the set of all rate constants appearing in the reduced system and in which $A_1,\ldots,A_R$ are assumed to be analytical functions of time.

These equations are obtained generally by invoking the *law of mass action principle*, and the constants k are understood to be specified in accord with collision-theoretic or absolute-rate-theoretic principles. The integral solutions of these equations, when they can be obtained, lead to ordinary (nonstochastic) analytic functions of time for each of the concentration random variables. The final form of the deterministic model would therefore consist of a set of $\sum_{i=1}^{N}(r_i + s_i)$ time functions which assign precise values to all of the concentration variables as functions of time; *i.e.*,

$$[{}^{(i)}A_{ji}] = {}^{(i)}\varphi_{ji}(t)\ (i = 1,2,\ldots,N)\ (j_i = 1,2,\ldots,r_i) \quad (3)$$

$$[{}^{(i)}B_{ki}] = {}^{(i)}\psi_{ki}(t)\ (i = 1,2,\ldots,N)\ (k_i = 1,2,\ldots,s_i) \quad (4)$$

Thus, once initial conditions are settled, the φ- and ψ- time functions allow the prediction of the concentration values of all reactants and products at any time, t, subsequent to the initiation of the reaction process. In practice, the possibility of fluctuations due to experimental error is allowed for by the addition of a random error variable, say ${}^{(i)}\epsilon_{ji}$ to equation (3) and ${}^{(i)}\delta_{ki}$ to equation (4). It is

[2] See Bartholomay (1960b) for a new mathematical representation of chemical equations and Bartholomay (1957) for a more detailed treatment of the material of this and the subsequent section.

generally assumed that these are normally distributed variates and the fit of data to a proposed mechanism is assigned statistical significance relative to the distributions of the ϵ and δ terms.

4. The Corresponding Stochastic Variables and Equations

The stochastic model for the general reaction mechanism (1), which represents a generalization of those already developed for a variety of reaction types, assumes that corresponding to each reactant species ${}^{(i)}A_{ji}$ is a *discrete random variable*, say ${}^{(i)}m_{ji}$, which has only a finite number of integral *states*. These *allowable states* range over the set of positive integers included between 0 and the maximal integral value $({}^{(i)}m_{ji})_0$. A "state" is defined as the number of molecules present per constant volume of reaction mixture. Thus the random variable associated with ${}^{(i)}A_{ji}$ has the allowable values

$$ {}^{(i)}m_{ji} = 0,1,2,3\ldots, ({}^{(i)}m_{ji})_0 \ (i = 1,2,3,\ldots,N) \quad (5) $$

Similarly, associated with the "product"-species ${}^{(i)}B_{ki}$ is a discrete-integral valued random variable ${}^{(i)}n_{ki}$, where

$$ {}^{(i)}n_{ki} = 0,1,2,3,\ldots, ({}^{(i)}n_{ki})_0, \ (i = 1,2,3,\ldots,N) \quad (6) $$

where now $({}^{(i)}n_{ki})_0$ is the maximally attainable value for that species.

The random variables are determined by the relative probabilities which at a given instant, t, may be assigned to all possible values. Thus, corresponding to the random variable ${}^{(i)}m_{ji}$, the calculation of the *probability function* $p({}^{(i)}m_{ji}, t)$ is required, where

$$ p\,({}^{(i)}m_{ji}, t) = \{p\,(0,t),p(1,t),p(2,t),\ldots, p\,({}^{(i)}m_{ji})_0,t)\} \quad (7) $$

$$ (\text{where} \sum_{x=0}^{({}^{(i)}m_{ji})_0} p\,(x,t) = 1 \text{ for all } t), \quad (8) $$

gives the probability of each of the possible values in (5) as a function of time. Similar expressions corresponding to the n's are required.

The complete specification of the stochastic model, however, calls for even more than the probability functions $p\ ({}^{(i)}m_{ji}, t)$ and $p\ ({}^{(i)}n_{ki}, t)$. It requires a knowledge of the interdependence of all random variables as expressed by the *multivariate* joint-probability density function.

For simplicity, in the remainder of the discussion it will be assumed that this large number $\sum_{i=1}^{N} (r_i + s_i) = \nu$ of random variables have been renamed $n_1,n_2,\ldots, n_\nu$. In these terms the stochastic process is completely defined if the joint-probability function

$$ p\,(n_1, n_2,\ldots,n_\nu; \mu; t) \quad (9) $$

is, in principle, determinable, where μ is a vector quantity whose components are all the "probability parameters" (*vide infra*) of the process.

As in the *Chapman-Kolmogorov* equations of Markovian processes (Doob, 1953; Bartholomay, 1957, 1958, 1959; Bharucha-Reid, 1960), the probability distribution (9) is not obtainable directly. It is subsumed as the solution of a stochastic *difference-differential* equation:

$$ \frac{\partial}{\partial t}\, p\,(n_1,n_2,\ldots,n_\nu; \mu; t) = f\,(n_1,n_2,\ldots,n_\nu; \mu, t) \quad (10) $$

where f is a polynomial function. By the *stochastic model* of the reaction process is meant either the difference-differential, equation (10), or its solution, as in expression (9). Thus, if either the deterministic or the stochastic theory is applied, time derivatives carry the specification of the kinetics. Comparison of equation (10) with equation (2) emphasizes that the deterministic theory is concerned with the rate of change of concentration; the stochastic theory, with rate of change of the *probabilities* of these concentrations; *i.e.*, with the flow of probabilities in the reaction time course.

5. Basic Probability Axioms of Construction

In order to obtain the distribution function, or at least its stochastic difference-differential equation, the following axioms already have been shown to lead to stochastic models which are "consistent in the mean" (Bartholomay 1957, 1958, 1959, 1961) relative to the corresponding deterministic equations (2), or (3) and (4). A specific illustration of the meaning of this criterion will be given in the sequel. The satisfaction of this criterion makes it highly likely that kinetic data which support a proposed mechanism formulated in deterministic terms will support the stochastic formulation as well, and *vice versa*.

A-1.—With any possible combination of different molecules in proportions specified by the stoichiometric coefficients on one side of a given step of the total system (1), *a priori* there is associated a (constant) probability parameter, say μ_i. This is an intrinsic and invariant characteristic of the reaction mechanism, and is the same for all such molecular combinations that can be formed at any time t. The parameter μ_i is such that $\mu_i\Delta t + 0(\Delta t)$ gives the probability that a given combination is actually transformed to the other side of the chemical equation in time $(t, t + \Delta t)$, where this expression is independent of t, Δt is an infinitesimal time interval, and $0(\Delta t)$ is a higher order infinitesimal such that $\lim_{\Delta t \to 0} \frac{0(\Delta t)}{\Delta t} = 0$.

Depending on the particulars of a given reaction, the parameters μ_i may be introduced in a variety of ways corresponding to different rate-theoretic partitionings of the deterministic rate constants (from which the quantity $\mu_i\,\Delta t + 0(\Delta t)$ is deducible with added physicochemical meanings). The second axiom has reference to this.

A-2.—The parameters μ_i of the stochastic model correspond to the rate constants, the k's of the deterministic model, and exhibit similar dependencies on the physical factors of the reaction and of its environment.

A-3.—The probability of more than one individual molecular transformation of a given kind is of the order of infinitesimal Δt, so that in the limit as $\Delta t \rightarrow 0$, this probability is negligible.

This axiom is an important determining factor in the final specification of the stochastic process. It corresponds to the simplest mathematical interpretation possible which leads to the kind of consistency just referred to. This, together with the linear dependence on Δt of the various probabilities such as $\mu_i \Delta t + 0(\Delta t)$ and axioms 4 and 5 below, makes possible a mathematically tractable combinatorial analysis of the events of reaction. Since, in the determination of the final distribution [expressions (9) or (10)] all of these statements are considered in the limit as $\Delta t \rightarrow 0$, this axiomatic requirement is not as stringent as would appear from considering time intervals of ordinary (non-infinitesimal) lengths Δt.

A-4.—All possible potentially effective combinations of molecules of the kind spoken of in axiom 1 are regarded *a priori* as "equally likely."

A-5.—Individual jointly occurring molecular transformation events are assumed to be statistically independent.

This axiom provides that the probability of a total reaction event made up of a number of jointly occurring events (*i.e.*, all occurring in an infinitesimal time interval $[t, t + \Delta t]$) is calculable by multiplying together the probabilities of all joint events.

Here, as in the deterministic treatment, interrelationships between the original random variables obtainable from the stoichiometry and initial conditions lead usually to a *reduced* or *canonical* set of random variables, say $\rho < \nu$ in number.

6. Stochastic Treatment of the Basic Michaelis-Menten (1913) Enzyme Kinetics

The specific action of an enzyme, E, on its substrate, S, initiates a *Bernoullian* (Feller, 1950) chain of random physical and chemical events culminating in the decomposition of substrate to products as the final event in the random chain. More specifically:

(*a*) The reaction process begins with random intermolecular collisions between molecules of all types promoted by the unpredictable erratic Brownian-like motions of the molecules in the reaction mixture. Thus, *a priori*, it is possible to assign a probability to the *effective* collision between an arbitrary pair of enzyme and substrate molecules. Moreover each such collision must occur randomly on the time-scale of the reaction in a manner which resembles the physicist's *Poisson law of rare events.*

(*b*) Once an *effective* collision between unlike molecules takes place, resulting in the formation of a complex enzyme-substrate molecule, this molecule intramolecularly passes in random fashion through a succession of various discrete energy level transitions describable in probabilistic terms as *random walks* (Feller, 1950; Doob, 1953). There are two possible eventual outcomes, corresponding to "absorbing barriers" in *random-walk theory*, with which there are associated probability weights: (1) either the complex molecule passes over the potential energy barrier and *decomposes* into the products of reaction, returning the enzyme molecule to its normal quantum range, or (2) the molecule falls back into the potential energy hole and dissociates, allowing the enzyme and substrate molecules both to return to the first stage of the random collision process.

(*c*) Clearly, if a long chain of intermediate forms, as in current theories of enzymatic mechanisms (Hearon, 1952; Lumry, 1955, 1959; Boeri, 1960), is conceived as separating the original reactants from the effective enzyme-substrate complex form, the randomness of the process is increased and, in greater detail, would be representable as a *chain process* (*with* or *without branching*).

Because of the random quality of the various events enumerated, the axioms of the stochastic kinetic theory can be applied to obtain an over-all stochastic model of the enzymatic reaction. The complexity of such a model depends on the complexity of the *probability-sample space* chosen. This is an arbitrary matter which is governed by the number of details of the random aspects of the process which makes for a feasible and mathematically tractable stochastic model. The least degree of complexity justifiable has been tried in most applications so far. In the present instance the number of probability parameters has been kept to a minimum, namely, three: μ_1, μ_2, and μ_3.

(*a*) *The "Association" Parameter* μ_1.—First of all, from the point of view of collision-theory, suppose that at time t in the progress of the reaction there are present n_1 molecules (per constant volume) of free enzyme E and n_2 molecules of substrate S, then the number of collisions between E and S molecules taking place in the interval $(t, t + \Delta t)$ equals (Strutt, 1912; Trautz, 1916; Lewis, 1918; Glasstone *et al.*, 1941; Tolman, 1950):

$$N_{12} = \left(\frac{8 \pi K T}{m_{12}}\right)^{1/2} d_{12}^2 \, n_1 \, n_2 \, \Delta t + 0(\Delta t) \qquad (11)$$

where $d_{12} = \frac{1}{2}(d_1 + d_2)$

d_1 = average diameter of an enzyme molecule
d_2 = average diameter of a substrate molecule
K = Boltzmann's constant
T = absolute temperature

$$m_{12} = \frac{m_1 \, m_2}{m_1 + m_2}$$

m_1 = average mass of enzyme molecule
m_2 = average mass of a substrate molecule

and where N_{12} is assumed to be taken to the nearest integer. The probability that a given collision which takes place in this time leads to an enzyme-substrate complex ES may be set equal to the very small constant $Pe^{-\epsilon/KT}$ where P is a constant and ϵ is the critical activation energy. This probability is taken to be invariant of the process.

Treating each of these collisions as a "Bernoulli trial" (Feller, 1950) with probability $Pe^{-\epsilon/KT}$ of "success" (*i.e.*, of complexation), and a probability, $1 - Pe^{-\epsilon/KT}$ of "failure," then the probability of formation of a single complex in this time is given by:

$$\binom{N_{12}}{1} (Pe^{-\epsilon/KT}) (1 - Pe^{-\epsilon/KT})^{N_{12}-1} \approx$$

$$Pe^{-\epsilon/KT} \left(\frac{8 \pi KT}{m_{12}}\right)^{1/2} d_{12}^2 n_1 n_2 \Delta t + 0(\Delta t), \quad (12)$$

a reasonable approximation if $Pe^{-\epsilon/KT}$ is "small" (where the first ( ) is the symbol for "combinations of"). The probability of formation of more than one such molecule in this time is negligible; this fact can be shown in similar fashion. *E.g.*, the probability of two such formations would be:

$$\binom{N_{12}}{2} (Pe^{-\epsilon/KT})^2 (1 - Pe^{-\epsilon/KT})^{N_{12}-2} \quad (13)$$

so that for small probability $Pe^{-\epsilon/KT}$, it is unnecessary to consider the possibility of more than one enzyme-substrate association over an infinitesimal time interval. The same effect may be achieved by postulating that all possible associations are to be treated not only as equally likely, *a priori*, but also as *mutually exclusive* in the probabilistic sense. In this case the law of addition of probabilities applied to the totality of N_{12} possible complexations leads to the same expression (12) for the probability of a single effective collision in the prescribed time. In any case, the collision-theoretic *probability parameter* μ_1 becomes the first part of the right-hand side of (12); *i.e.*, by definition,

$$\mu_1 \equiv Pe^{-\epsilon/KT} \left(\frac{8 \pi KT}{m_{12}}\right)^{1/2} d_{12}^2 \quad (14)$$

Accordingly, the probability of complexation in time $(t, t + \Delta t)$ will be written more conveniently as

$$[\mu_1 n_1 n_2 \Delta t + 0(\Delta t)]. \quad (15)$$

Alternatively (Bartholomay, 1961), the basic parameter μ_1 may be introduced without any specific physical connotation; *i.e.*, axiomatically, as in axiom A-1, without deriving it from collision theory. Once expression (15) is obtained in this fashion it may of course be repartitioned along the lines of *collision-theory* or, in fact, of *absolute rate theory* (Bartholomay, 1960a).

The simplest probability space and most general expression results from the direct axiomatic procedure, which is deducible as follows. If at time t there are n_1 molecules of free enzyme and n_2 molecules of substrate, then there are $n_1 n_2$ possible pairings, each of which is a potential enzyme-substrate complex. According to the axioms, an arbitrarily small (infinitesimal) interval of length Δt is considered so that in the subsequent interval $(t, t + \Delta t)$ no more than one of these will be realized. Thus $n_1 n_2$ mutually exclusive random events are to be considered. If $\mu_1 \Delta t + 0(\Delta t)$ represents the probability (the same for each pairing) that any given pairing becomes an actual complex in this time, then $n_1 n_2 \mu_1 \Delta t + 0(\Delta t)$ is the probability of obtaining one such conversion in this time.

(*b*) *The Dissociation Parameter* μ_2. As in the general stochastic model for unimolecular reactions (Bartholomay, 1957, 1958, 1959; Bharucha-Reid, 1960), it is reasonable to suppose that if n_3 enzyme-substrate molecules are present at time t, in a subsequent infinitesimal interval $(t, t + \Delta t)$ each of the n_3 molecules has the same probability $\mu_2 \Delta t + 0(\Delta t)$ of dissociation. Again these can be treated as mutually exclusive events, in which case the probability of a single dissociation in time $(t, t + \Delta t)$ would be $\mu_2 n_3 \Delta t + 0(\Delta t)$, and that of more than one dissociation would be of the order of Δt, according to the axioms.

(*c*) *The Decomposition Parameter* μ_3. In similar fashion, the probability of securing a decomposition in time $(t, t + \Delta t)$ from the n_3 molecules present at time t is taken to be $\mu_3 n_3 \Delta t + 0(\Delta t)$.

The parameters μ_2, μ_3 may be put on a similar collision-theoretic basis as μ_1, say, by invoking the *Lindemann-Hinshelwood* (Lindemann, 1922; Hinshelwood, 1927) mechanism of first-order kinetics, so that in all cases the parameters exhibit the same kind of dependency as their deterministic counterparts, the rate constants.

The derivation of the stochastic difference-differential equation of the process:

$$\frac{\partial}{\partial t} p(n_1,n_2,n_3,n_4^*;\ \mu_1,\mu_2,\mu_3;\ t) =$$

$$f(n_1,n_2,n_3,n_4;\ \mu_1,\mu_2,\mu_3;\ t) \quad (16)$$

* n_4 is the number of molecules of product P formed.

is considered next.

This can be simplified by taking advantage of interdependencies (*vide infra*) between the four variables, which reduce the last equation to:

$$\frac{\partial p(n_2 n_3;\ t)}{\partial t} = f(n_2 n_3;\ \mu_1,\mu_2,\mu_3;\ t) \quad (17)$$

(The μ's will be omitted subsequently in probability expressions for convenience in writing.) Thus, where n_{i0} $(i = 1,2,3,4)$ is the initial concentration of each substance involved,

$$\left.\begin{aligned} n_1 &= n_{10} - n_3 \\ n_4 &= n_{20} - (n_2 + n_3) \end{aligned}\right\} \quad (18)$$

By means of equations (18) all expressions involving n_1 and n_4 can be rewritten in terms of the two "canonical" random variables n_2 and n_3. The ranges of the various variables are the following:

$$0 \leqslant n_1 \leqslant n_{10} \ll n_{20} \quad (19)$$

$$0 \leqslant n_2 \leqslant n_{20} \quad (20)$$

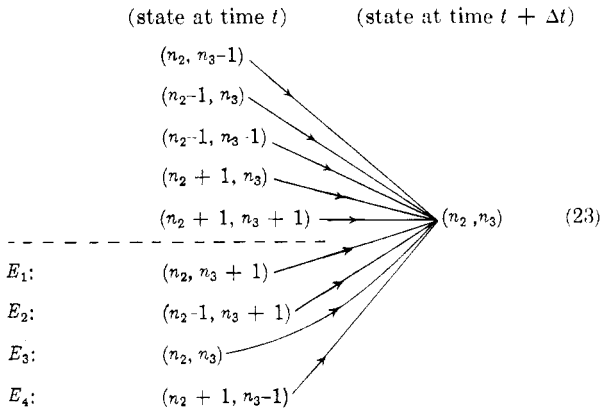
$$0 \leqslant n_3 \leqslant n_{10} \quad (21)$$

$$0 \leqslant n_1 \leqslant n_{10} \quad (22)$$

In setting up the difference-differential equation first an expression for $[p(n_2,n_3;\ t + \Delta t) - p(n_2,n_3;\ t)]$ is obtained indirectly. Then this is divided by Δt. The resulting difference-quotient passes to a limit

$\frac{\partial p}{\partial t}$ $(n_2,n_3;\ t)$ as the infinitesimal Δt is made to approach zero.

To obtain an expression for $[p\ (n_2,n_3;\ t + \Delta t) - p\ (n_2,n_3;\ t)]$ it is necessary to assign probabilities to the various *transitions* which, starting at time t can lead to values n_2,n_3 at time $t + \Delta t$. Since the assumptions made dictate that these variables can either decrease by one, increase by one, or remain fixed in this time, *a priori* there are nine states allowable at time t which could lead to the values n_2 and n_3 of the substrate, enzyme-substrate variables Δt later. In other words, the following transitions have to be considered:



(23)

The first five can be ruled out as having negligible probabilities (Bartholomay, 1961), but the remaining four transitions represent mutually exclusive events which can lead to the state (n_2,n_3) at time $t + \Delta t$. It is therefore necessary to obtain detailed expressions for the probabilities of E_1, E_2, E_3, E_4. The derivation of the first of these only will be given; $P\ (E_1) = Pr\ \{n_2,n_3;\ t + \Delta t | n_2,n_3 + 1;\ t\}$: the conditional probability that if the random variables involved have values n_2, $n_3 + 1$ at time t, then they have the required values n_2, n_3 at time $t + \Delta t$.

The event E_1 comprises the joint occurrence of four independent composite events E_{11}, E_{12}, E_{13}, E_{14}, the probabilities for which may be expressed in terms of the over-all probability function $p\ (n_2,n_3;\ t)$ as follows: (1) E_{11}: the presence of n_2 molecules of substrate and $n_3 + 1$ molecules of enzyme-substrate.

$$P\ (E_{11}) = p\ (n_2,n_3 + 1;\ t) \qquad (24)$$

(2) E_{12}: the decomposition of one molecule of ES in time $(t,\ t + \Delta t)$ (implying the joint transitions: $n_3 + 1 \rightarrow n_3$, $n_1 - 1 \rightarrow n_1$, $n_4 - 1 \rightarrow n_4$).

$$P\ (E_{12}) = (n_3 + 1)\ \mu_3\ \Delta t + 0(\Delta t) \qquad (25)$$

(3) E_{13}: no additional enzyme-substrate complex formation in time $(t,\ t + \Delta t)$.

Since at time t there were $n_1 - 1$ molecules of E and n_2 of S, the probability of the formation of a complex in this time equals $(n_1 - 1)\ n_2\ \mu_1\ \Delta t + 0(\Delta t)$. Hence the probability against this, $P\ (E_{13})$, is given by

$$P\ (E_{13}) = 1 - (n_1 - 1)\ n_2\ \mu_1\ \Delta t + 0(\Delta t), \qquad (26)$$

$$\text{or } P\ (E_{13}) = 1 - (n_{10} - n_3 - 1)\ n_2\ \mu_1\ \Delta t + 0(\Delta t) \qquad (27)$$

(4) E_{14}: No dissociation of the existing complexes. Thus

$$P\ (E_{14}) = 1 - (n_3 + 1)\ \mu_2\ \Delta t + 0(\Delta t) \qquad (28)$$

Therefore,

$$P\ (E_1) = \prod_{i=1}^{4} P\ (E_{1i}) =$$

$$p\ (n_2,n_3 + 1;\ t)\cdot(n_3 + 1)\ \mu_3 \Delta t + 0(\Delta t) \qquad (29)$$

Similarly,

$$P\ (E_2) = Pr\{n_2,n_3;\ t + \Delta t | n_2 - 1,\ n_3 + 1;\ t\} =$$

$$p\ (n_2 - 1,\ n_3 + 1;\ t)\ (n_3 + 1)\ \mu_2\ \Delta t + 0(\Delta t) \qquad (30)$$

$$P\ (E_3) = Pr\ \{n_2,n_3;\ t + \Delta t | n_2,n_3;\ t\} =$$

$$p\ (n_2,n_3,\ t)[1 - (n_{10} - n_3)\ n_2\ \mu_1\ \Delta t -$$

$$(\mu_2 + \mu_3)\ n_3\ \Delta t + 0(\Delta t)] \qquad (31)$$

$$P\ (E_4) = Pr\ \{n_2,n_3;\ t + \Delta t | n_2 + 1,\ n_3 - 1;\ t\} =$$

$$p\ (n_2 + 1,\ n_3 - 1;\ t)\ [\mu_1\ (n_{10} - n_3 + 1)$$

$$(n_2 + 1)\Delta t + 0(\Delta t)] \qquad (32)$$

Since these mutually exclusive events E_1, E_2, E_3, E_4 exhaust all possible alternatives for arriving in time $(t,\ t + \Delta t)$ at $(n_1,\ n_2,\ n_3,\ n_4)$; or simply, (n_2,n_3):

$$p\ (n_2,n_3;\ t + \Delta t) = \sum_{j=1}^{4} P\ (E_j) =$$

$$p\ (n_2,n_3 + 1;\ t)\ (n_3 + 1)\ \mu_3\ \Delta t +$$

$$p\ (n_2 - 1,\ n_3 + 1;\ t)\ (n_3 + 1)\ \mu_2\ \Delta t +$$

$$p\ (n_2,n_3;\ t)[1 - (n_{10} - n_3)\ n_2\ \mu_1 - \Delta t$$

$$(\mu_2 + \mu_3)n_3\ \Delta t] + p\ (n_2 + 1,\ n_3 - 1;\ t)$$

$$[(n_{10} - n_3 + 1)(n_2 + 1)\ \mu_1\ \Delta t] + 0(\Delta t) \qquad (33)$$

Subtracting $p\ (n_2,n_3;\ t)$ from both sides of this expression, dividing by Δt, and passing to the limit as $\Delta t \rightarrow 0$ gives finally the *stochastic difference-differential equation of the basic enzymatic reaction:*

$$\frac{\partial p(n_2,n_3;\ t)}{\partial t} = \mu_3(n_3 + 1)\ p\ (n_2,n_3 + 1;\ t) +$$

$$\mu_2(n_3 + 1)\ p\ (n_2 - 1,\ n_3 + 1;\ t) -$$

$$n_1\mu_2(n_{10} - n_3)\ p\ (n_2,n_3;\ t) - (\mu_2 + \mu_3)n_3\ p\ (n_2,n_3;\ t) +$$

$$\mu_1\ (n_2 + 1)\ (n_{10} - n_3 + 1)\ p\ (n_2 + 1,\ n_3 - 1;\ t) \qquad (34)$$

This, in turn, may be shown to be equivalent to the following second-order partial differential equation of the process:

$$\frac{\partial \varphi}{\partial t} = (\mu_3 + \mu_2\ s_2 - \mu_2\ s_3 - \mu_3\ s_3)\ \frac{\partial \varphi}{\partial s_3} +$$

$$n_{10}\ \mu_1\ (s_3^2 - s_2)\ \frac{\partial \varphi}{\partial s_2} + \mu_1\ s_3\ (s_2 - s_3)\ \frac{\partial 2\varphi}{\partial s_2\ \partial s_3} \qquad (35)$$

where $\varphi\ (s_2,s_3,t) = \sum_{n_2=0}^{n_{20}} \sum_{n_3=0}^{n_{10}} p(n_2,n_3;\ t)\ s_2^{n_2} s_3^{n_3}$ is the "probability generating function" of the process and s_2, s_3 arbitrary variables.

7. Comparison with the Classical Deterministic Theory

Either of these equations is at the least equivalent in information value to the system of differential equations which comprise the classical deterministic model underlying the Michaelis-Menten or Briggs-Haldane theory, with which they may be compared. Corresponding to the Michaelis-Menten system of stoichiometric equations[2]

$$\left.\begin{array}{l} E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \\ ES \xrightarrow{k_3} E + P \end{array}\right\} \qquad (36)$$

The system of differential equations is as follows:

Classical ordinary differential equations of the basic enzymaic reaction

$$\frac{d[E]}{dt} = -k_1[E][S] + (k_2 + k_3)[ES] \qquad (37)$$

$$\frac{d[S]}{dt} = k_2[ES] - k_1[E][S] \qquad (38)$$

$$\frac{d[ES]}{dt} = k_1[E][S] - (k_2 + k_3)[ES] \qquad (39)$$

$$\frac{d[P]}{dt} = k_3[ES] \qquad (40)$$

In particular, the pair (38), (39) is referred to as the system of *canonical equations* (Bartholomay, 1960a). Neither in this case nor in the stochastic case has a general integral solution been obtained. However, the consistency between the stochastic and the classical theory may be demonstrated as follows.

The *average* or *mean* values of the random variables n_2 and n_3 with respect to the over-all distribution function are by definition

$$\overline{n_2(t)} = \sum_{n_2=0}^{n_{20}} \sum_{n_3=0}^{n_{10}} n_2\, p\,(n_2,n_3;t) \qquad (41)$$

$$\overline{n_3(t)} = \sum_{n_2=0}^{n_{20}} \sum_{n_3=0}^{n_{10}} n_3\, p\,(n_2,n_3;t) \qquad (42)$$

Differentiating with respect to time gives

$$\frac{d\bar{n}_2}{dt} = \sum_{n_2=0}^{n_{20}} \sum_{n_3=0}^{n_{10}} n_2 \frac{\partial p\,(n_2,n_3;t)}{\partial t} \qquad (43)$$

$$\frac{d\bar{n}_3}{dt} = \sum_{n_2=0}^{n_{20}} \sum_{n_3=0}^{n_{10}} n_3 \frac{\partial p\,(n_2,n_3;t)}{\partial t} \qquad (44)$$

Substitution of expression (34) into (43) and (44), after algebraic rearrangements, leads eventually to:

$$\frac{d\bar{n}_2}{dt} = \mu_2 \sum_{n_2=0}^{n_{20}} \sum_{n_3=0}^{n_{10}} n_3\, p(n_2,n_3;t) - \mu_1 \sum_{n_2=0}^{n_{20}} \sum_{n_3=0}^{n_{10}} (n_{10} - n_3) n_2\, p(n_2,n_3;t) \qquad (45)$$

$$\frac{d\bar{n}_3}{dt} = \mu_1 \sum_{n_2=0}^{n_{20}} \sum_{n_3=0}^{n_{10}} (n_{10} - n_3)\, n_2\, p(n_2,n_3;t) - (\mu_2 + \mu_3) \sum_{n_2=0}^{n_{20}} \sum_{n_3=0}^{n_{10}} n_3 p(n_2,n_3;t) \qquad (46)$$

[2] k_2 here corresponds to k_{-1} in the general formulation of section 3; and k_3 here to k_2.

which amount to:

$$\frac{d\bar{n}_2}{dt} = \mu_2 \bar{n}_3 - \mu_1 \overline{n_1 n_2} \qquad (47)$$

$$\frac{d\bar{n}_3}{dt} = \mu_1 \overline{n_1 n_2} - (\mu_2 + \mu_3)\bar{n}_3 \qquad (48)$$

The forms of these equations are exactly those of the deterministic canonical equations. In fact, substituting for μ_1,μ_2,μ_3 the constants k_1,k_2,k_3, and, for the stochastic mean values $\bar{n}_3$ and $\overline{n_1 n_2}$, the ordinary deterministic concentration variables $[ES]$, $[E][S]$ reduces (47) and (48) to equations (38) and (39) of the deterministic model.

9. General Discussion

The stochastic model for Michaelis-Menten kinetics obtained here illustrates the meaning of the "consistency in the mean" criterion mentioned earlier. Because of the agreement of the central tendency of the stochastic process with the deterministic time course, it follows that data which support the deterministic equations of the Michaelis-Menten theory will also support the stochastic model. In addition, the stochastic model provides a more appropriate mathematical framework for the probabilistic aspects of kinetic theories such as the collision theory and the modern absolute rate theory. It raises the question of the meaning of "reproducibility" in dealing with reaction mechanisms by demonstrating mathematically that the randomness associated with initial inter- and intramolecular events must be taken into account in considering the eventual time course of the concentration states of a reaction process. Consequently, *inherent irreproducibility* should be considered alongside, but independently of, the imprecision of experimental methods (or *experimental irreproducibility*) in judging the value of a method and the fit of a theoretically proposed biochemical mechanism to data.

According to the stochastic theory, then, even discounting experimental error, kinetic data obtained in a single experimental run will always exhibit random fluctuations around the central tendency of the reaction process; they describe a *sample curve* of the reaction process. The most careful statistical analysis of data consequently would have to take into account the extent of inherent irreproducibility. As the mathematical theory for such processes develops further along numerical lines, expressions may be found for the extent of inherent variation to be expected. Since this cannot be determined experimentally, further progress rests on further numerical analysis and extensive computation using the formulas given. At the same time it should be emphasized that the difficulty of the mathematical situation is really no worse here than in the classical theory introduced half a century ago. A closed solution of the canonical system of equations (38) and (39) has never been obtained, and the field has had to develop along approximate lines. Because of the corre-

spondences between the classical and the stochastic formulation there are really common mathematical problems to be solved.

Since the classical equations are deducible from the stochastic equations, and not conversely, the stochastic equations contain more information. This is evident from the fact that the classical non-stochastic theory allows one to say nothing of fluctuations whatever—probabilistic considerations lie outside of the realm of the theory. Thus the stochastic approach is much more congenial with the methods of theoretical physics which have been brought to bear on reaction rate theories already.

Furthermore, passage to the limit, as $t \rightarrow \infty$, of the total distribution of the process allows one to envisage a kinetic approach to the equilibrium state using the same mathematical considerations which underly the statistical thermodynamics of the equilibrium state—so that there arises the possibility of a general homogeneous statistical theory for treating the entire reaction time course.

While the present stochastic equations have not been studied extensively enough to demonstrate the point, previous stochastic studies (Bartholomay, 1957) of the simple reversible process $A \underset{k_2}{\overset{k_1}{\rightleftharpoons}} B$ have led to the derivation of the probability distributions of A and B at equilibrium; *i.e.*, the definition of a *stochastic equilibrium state* which corresponds in value to the ordinary equilibrium concentration specified by the deterministic theory. The variance of the distribution at equilibrium of the random variable n corresponding to species A was shown (Bartholomay, 1957) to equal $\frac{\mu_1\mu_2\, n_0}{(\mu_1 + \mu_2)^2}$ where μ_1,μ_2 are the stochastic parameters of the process and n_0 the initial value of n. Thus if $\mu_1 = \mu_2$, the standard deviation of the equilibrium state would be $^1/_2\sqrt{n_0}$ (in agreement with the "$\sqrt{n}$ law," *vide infra*). According to this theory, then, it is more accurate to speak of a whole *range* of equilibrium values, as opposed to a single value.

Finally it should be remarked that the connection noted between the stochastic equations and the deterministic equations (*i.e.*, the latter is the average value of the former) has been found to hold in other chemical reactions studied as well (Bartholomay, 1957–1959; Bharucha-Reid, 1960). Since, in the derivation of the stochastic equations, the law of mass action has not been invoked as a starting point, and because of the *mean-consistency* of stochastic with deterministic results obtained by application of the mass action law, it can be considered that the general formulation proposed amounts to a statistical reformulation of the law of mass action. This point of view is consistent with general comments made by Schroedinger (1945) on the approximate nature of scientific laws. In fact, the orders of magnitude of various stochastic distributions (such as the equilibrium distribution mentioned in the previous paragraph) obtained agrees with his "$\sqrt{n}$ law of error."

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