BULLETIN OF
MATHEMATICAL BIOPHYSICS
VOLUME 16, 1954

STUDIES ON THE EFFECTS OF STRUCTURE ON THE BEHAVIOR OF ENZYME SYSTEMS

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The effects of structural features on various properties of enzyme systems are studied. Some of the effects are: in a homogeneous reaction, enzyme compartmentalization decreases the rate; in a heterogeneous reaction, compartmentalization increases the rate. The steady-state concentration of intermediates is larger in a non-uniform than in uniform systems. Periodicities do not generally occur in the common kinetic systems; they do occur in autocatalytic systems, but compartmentalization reduces their probability of occurrence. The conditions for overshoot are different for uniform and non-uniform systems. Multiple stable steady states are not a common occurrence among biologically typical reactions; they do occur in combined autocatalytic and surface systems (a mechanism for the gene position effect is suggested by this property). The local pH is affected by the enzyme aggregation as well as by the geometry of the enzyme structure. A 2-step system can give rise to the characteristic rate vs. pH curve, where the optimum is not necessarily at isoelectric point. The expression for the osmotic pressure inside a spherical particle is deduced. The pressure is shown to be dependent on the radius. The rate inside a cell particle is shown to be determined by the shape of the particle.

Biologists have recognized for some time that a living system (cell, organ, organism) is not simply a homogeneous volume in which chemical reactions proceed. It represents, rather, a structure of definite spatial organization, which organization is of decisive importance in determining the behavior of the cell. This fact has received additional recognition in view of recent developments in cytochemistry, where it has been shown that mitochondria are the seat of whole enzyme systems in a large variety of organisms, ranging from bacteria (Mudd, 1953) to insects (Levenbook, 1953), plants (Bonner and Millerd, 1953), and mammals (Hogeboom and Schneider, 1950; Green, 1952). In particular, the mitochondria contain the enzymes of the all important tricarboxylic acid cycle (Kennedy and Lehninger, 1949) as well as of oxidative phosphorylation (Potter, Lyle, and Schneider, 1951), and the activity of these enzymes depends decisively on the spatial integrity of the mitochondria (Green, *loc. cit.*).

Additional evidence concerning the existence of structural organization in cellular processes can be found in various areas of biology. We can mention here, among others, the localization of ribose nucleic acid in micro-

somes (Holter, 1952) which may imply the localization of protein synthesis. The localization in cellular membranes of enzymes participating in the transport of metabolites is also noteworthy (Lindberg, 1950; Rothstein, Meier, and Hurwitz, 1951).

Another aspect worthy of mention is the discovery (Harman, 1950) of a definite correlation between the changes in the shape of mitochondria and alterations in the general physiological state of the cell. Here the old problem of the relation of structure to function has reappeared on the microscopic level, linked most intimately with some of the most important metabolic processes.

It is interesting to consider the possible effects such structures may have on the behavior of the cell. In certain specific cases, as in the preservation of bioelectric potential or in active transport (Bierman, 1953), the function of the structural elements can be fairly easily postulated. But in dealing with more general metabolic systems other, more general effects need to be considered. In a recent stimulating book, D. Danielli (1950) discussed some of the variables which are of significance in controlling enzyme systems. He listed the following factors: pH, sulfhydryl (SH) concentration, concentrations of inhibitors and activators, and those processes controlling access of substates to enzymes.

In this paper the following problems will be considered:

1. The effect of enzyme localization upon the steady-state rate of a reaction sequence.
2. The effect of enzyme localization on concentration of reaction intermediates.
3. The influence of enzyme localization on competing substrates.
4. The effect of enzyme localization on the manner by which the steady state is reached, e.g., oscillations and overshoot.
5. The effect of structures on the possibility of multiple steady states.
6. The effect of enzyme localization of the local pH.
7. The effect of enzyme localization on osmotic pressure.
8. Some relation between the shape of the cell particle and the steady-state rate.

It is hoped that the consideration of these problems will lead to a more concrete evaluation of the role of structured enzyme systems.

Before proceeding, we must specify what shall be considered a structured enzyme system. If we consider cellular particles (mitochondria) two possibilities arise:

1. The enzymes can be distributed uniformly inside the particle (most probably in soluble form).

2. The enzymes can be fixed in specific regions or compartments of the particle.

For our purposes the significant difference lies in the following. In the first case, the "uniform" system, each step of a reaction sequence is carried out in the same volume and hence diffusion is ignored. In the second case, the "non-uniform" system, different steps occur in different regions and hence products of one reaction must diffuse from the region of their production to the region of their consumption for the reaction to be able to proceed. On the basis of present experimental evidence (Palade, 1953) both types seem to exist. If the enzymes are uniformly distributed, this type of localization differs from the non-localized system (e.g., the enzymes of anaerobic glycolysis) in that the reaction is compressed into a small volume and the intermediates are able to diffuse into and out of the reaction volume. The first model of a structured system is, therefore, a system in which all enzymes are uniformly distributed, but in which intermediates diffuse. Attention must be paid here to the problem of volume dependence of reaction rates.

The second, "non-uniform," model is that of a volume divided up into various compartments, i.e., regions in which enzymes are localized. As described before, for a reaction sequence to occur, substrates must diffuse from one such compartment to the next. This picture of a sequence of compartments conforms to recent photographic evidence concerning the internal structure of mitochondria (Palade, *loc. cit.*). Electron microscope investigation has shown that mitochondria are in all probability divided up lengthwise into segments or cristae, which could very well be loci of enzymes.

These compartments may be so arranged that each enzyme is adjacent to the one following it in the reaction sequence, and, furthermore, are close enough that each product is simply "handed over" from one catalytic surface to the other. This is the picture biochemists frequently have in mind when they speak of enzyme localization (Michaelis, 1951). For these reactions, the advantage of enzyme localization is apparent. On the other hand, it requires a structure extremely well adapted to one particular reaction sequence; that is, if the reaction proceeds in the order of intermediates, X_1, X_2, ... X_n, the enzymes have to be located precisely in that order.

We shall consider the possibility that the enzymes are not quite so pre-

cisely ordered, and that they are separated by some distance over which the substrates must diffuse. Since the distances involved are quite small, on the order of hundreds of Angstroms, a fairly random arrangement of compartments would be quite acceptable.

We shall now proceed to examine the criteria listed above to determine the effect of enzyme localization on cellular metabolism and behavior.

I. Steady-state rates. It is clear that one of the most significant properties to be examined is the steady-state rate of biological reactions. Such studies have been carried out for open and closed systems (Hearon, 1949*a*, *b*), but always on the assumption that the enzymes are uniformly distributed throughout the reaction volume. We shall consider here the particular effect of enzyme localization on the steady-state rate.

Two comments need to be made concerning the model which we shall investigate. First of all, the volumes in which the reactions take place (mitochondria, etc.) occur in the cytoplasm, and it is this cytoplasm which makes up their external environment. We shall treat this environment as uniform and unchanging in time. Further, wherever experiments are suggested involving manipulation of the "external" concentrations of metabolites, we have in mind particles that have been separated from the cellular matrix by centrifugation and whose environment is now directly accessible to experimental control. Secondly, throughout this first section we shall treat reactions of the type

$$C_j + X_{j-1} \underset{k_{-j}}{\overset{k_j}{\rightleftharpoons}} B_j + X_j , \qquad j = 1, 2, \ldots n . \quad (1)$$

The C_j's, X_j's, X_{j-1}'s, and B_j's are all considered as substrate and product molecules. *The enzymatic effect is included in the coefficients* k_j *and* k_{-j}. This approximation is, of course, only valid as long as the enzymes are not saturated, i.e., as long as the reaction rate is linear with respect to the enzyme concentration. Hence, if so desired, we can also think of k_j and k_{-j} as $\nu_j E_j$ and $\nu_{-j} E_{-j}$ respectively, where ν_j and ν_{-j} are constants and E_j and E_{-j} the concentrations of the enzymes of the jth step.

A. Uniform system. The first model, that of a uniformly distributed enzyme system inside a cell particle, differs only from the commonly described open system reactions in that intermediates are permitted to diffuse. This case was treated very briefly by Hearon (*loc. cit.*), and we shall investigate it here more fully.

Consider a reaction system as described in (1), where the concentrations C_j and B_j, as well as X_0 and X_n, are considered to be constant with respect to time, while the concentrations of the intermediates X_1 through X_{n-1} are

functions of time. Let $G(X_j)$ represent the flow rate of the jth intermediate per unit volume out of the reaction chamber. By definition, the rates per unit volume of the jth and $(j+1)$th step are given by

$$\left.\begin{aligned} v_j &= k_j C_j X_{j-1} - k_{-j} B_j X_j \,, \\ v_{j+1} &= k_{j+1} C_{j+1} X_j - k_{-(j+1)} B_{j+1} X_{j+1} \end{aligned}\right\} . \tag{2}$$

In the steady state dX_j/dt must equal zero. Hence

$$k_j C_j X_{j-1} - k_{-j} B_j X_j - k_{j+1} C_{j+1} X_j + k_{-(j+1)} B_{j+1} X_{j+1} - G(X_j) = 0 \,. \tag{3}$$

It follows from (2) and (3) that

$$v_{j+1} = v_j - G(X_j) \,,$$

or, more generally,

$$v_{j+m} = v_j - \sum_{k=j}^{j+m-1} G(X_k) \,. \tag{4}$$

The meaning of (4) is clear. Given a system from which intermediates diffuse, *different steps in the reaction sequence will have different steady-state rates.* The relation between the steady-state rate of the $(j+m)$th and the jth step is given by (4).

Our problem now is to investigate the effect of this diffusion of intermediates on the steady-state rate. We shall limit our investigation to the 2-step system, because of the mathematical complexity involved in the solution of equations representing higher step systems. The system studied here is the same as that investigated by previous workers in the field (Hearon, *loc. cit.*) and can be represented as follows:

$$\left.\begin{aligned} C_1 + X_0 &\rightleftharpoons X_1 + B_1 \,, \\ X_1 + C_2 &\rightleftharpoons X_2 + B_2 \,. \end{aligned}\right\} \tag{5}$$

Following our previous notation let $w_i \equiv k_i C_i, w_{-i} \equiv k_{-i} B_i$. As stated before, X_1 is the only metabolite whose concentration is a function of time. In particular, we shall assume that it is kept at a fixed concentration X_{10} outside of the reaction system, and diffuses out of the system at the rate $\lambda(X_1 - X_{10})$ per unit volume. Consider a reaction chamber of volume $2V$, surface area $2A$, and concentration X_1. If D is the diffusion coefficient of X_1, the outflow per unit volume is given by

$$\frac{2AD}{2V\Delta l_1}(X_1 - X_{10}) \,,$$

where Δl_1 is an element of length of the order of magnitude of a particle diameter (Rashevsky, 1948). Hence $\lambda = DA/V\Delta l_1$. We shall discuss this more fully later on.

We can now find the value of the steady-state rate of the first step, $v_1 = w_1X_0 - w_{-1}X_1$. Setting (3) equal to zero and remembering $G(X_1) = \lambda(X_1 - X_{10})$, we find

$$X_1 = \frac{w_1X_0 + \lambda X_{10} + w_{-2}X_2}{w_{-1} + w_2 + \lambda}. \tag{6}$$

Inserting (6) into the expression for v_1 gives us

$$v_1 = \frac{w_1X_0(\lambda + w_2) - w_{-1}w_{-2}X_2 - w_{-1}\lambda X_{10}}{\lambda + w_2 + w_{-1}}. \tag{7}$$

If we designate by q the corresponding rate in the non-diffusing system ($\lambda = 0$), the ratio q/v_1 becomes

$$\frac{q}{v_1} = 1 + \frac{w_{-1}\lambda\,[X_{10}(w_2 + w_{-1}) - w_{-2}X_2 - w_1X_0]}{(w_2 + w_{-1})\,[w_1X_0(\lambda + w_2) - w_{-1}w_{-2}X_2 - w_{-1}\lambda X_{10}]}. \tag{8}$$

We shall also assume that in the non-structured system the 2-step chemical reaction derived above proceeds in the forward direction. This requires that the change in free energy of that system be negative. This means that

$$RT \ln \frac{w_{-1}w_{-2}X_2}{w_1w_2X_0}$$

is negative, or $w_1w_2X_0 > w_{-1}w_{-2}X_2$. It can be seen from (8) therefore that q/v_1 is less than 1 for $X_{10} = 0$, becomes 1 if

$$X_{10} = \frac{w_1X_0 + w_{-2}X_2}{w_2 + w_{-1}},$$

and approaches infinity at

$$X_{10} = \frac{w_1X_0(\lambda + w_2) - w_{-1}w_{-2}X_2}{w_{-1}\lambda}.$$

For larger values of X_{10}, q/v_1 is always less than 1 (Appendix 1A). Hence variations in the external intermediate concentration result in wide variations in the value of the above ratio.

From (4) and (6) and remembering the definition of $G(X_1)$, we can solve for v_2. The expression is

$$v_2 = \frac{w_1w_2X_0 - w_{-1}w_{-2}X_2 - \lambda w_{-2}X_2 + \lambda w_2X_{10}}{\lambda + w_2 + w_{-1}}. \tag{9}$$

Hence q/v_2 ($q = v_2$ when $\lambda = 0$) is given by

$$\frac{q}{v_2} = 1 + \frac{w_2\lambda\,[w_1X_0 + w_{-2}X_2 - X_{10}(w_2 + w_{-1})]}{(w_2 + w_{-1})\,[w_1w_2X_0 + \lambda w_2X_{10} - w_{-2}X_2(w_{-1} + \lambda)]}. \tag{10}$$

A number of observations can be made regarding (10).

1. If X_{10} is zero, *and*

$$\lambda < \frac{w_1 w_2 X_0}{w_{-2} X_2} - w_{-1}, \tag{11}$$

the ratio is greater than 1. Considering that

$$\Delta F = RT \ln \frac{w_{-1} w_{-2} X_2}{w_1 w_2 X_0}, \tag{12}$$

the second condition can also be written $\lambda < w_{-1}(e^{-(\Delta F/RT)} - 1)$.

If this second condition is not fulfilled, i.e., if the flow coefficient λ is large, this ratio becomes less than 1.

2. The ratio becomes 1 when

$$X_{10} = \frac{w_1 X_0 + w_{-2} X_2}{w_2 + w_{-1}}$$

and is less than 1 for larger X_{10}. It should be pointed out that q/v_1 equals 1 for the same value of X_{10} as q/v_2.

The effects of large X_{10} and large λ are therefore the same: to increase v_2 relative to q.

Systems such as described do, of course, occur in cellular particles, and it would seem possible, at least in principle, to measure the two rates, v_1 and v_2, independently (by measuring, for example, the rates of appearance of B_1 and B_2). The ratio of these two rates is given by

$$\frac{v_2}{v_1} = 1 + \frac{\lambda [X_{10}(w_{-1} + w_2) - w_1 X_0 - w_{-2} X_2]}{w_1 \lambda X_0 + X_0 w_1 w_2 - w_{-1} w_{-2} X_2 - w_{-1} \lambda X_{10}}. \tag{13}$$

This ratio can be plotted as a function of X_{10} (Fig. 1). Experimentally, four measurements should be possible:

1. v_2/v_1 when X_{10} equals zero,
2. the value of X_{10} when v_2/v_1 equals 1,
3. the value of X_{10} when v_1 equals zero, and
4. the asymptotic value of v_2/v_1 as X_{10} becomes very large.

If we assume that the second step is irreversible ($w_{-2} = 0$) and designate the experimental values of the four measurements above by Q_1 through Q_4, the following ratios can be easily found

$$\left.\begin{aligned} \frac{w_2}{\lambda} &= \frac{Q_1}{1 - Q_1}, \\ \frac{w_2}{w_{-1}} &= -Q_4, \\ \frac{w_{-1}}{\lambda} &= \frac{Q_1}{Q_4 (Q_1 - 1)}. \end{aligned}\right\} \tag{14}$$

Also, as a test of the validity of the model, the following relation must hold between the Q's

$$Q_2 = \frac{Q_3(1-Q_1)}{1-Q_4}. \tag{15}$$

B. The non-uniform system. We have defined the non-uniform system to be a reaction volume, with diffusible intermediates, which is distinguished from the uniform system in that it consists of a number of com-

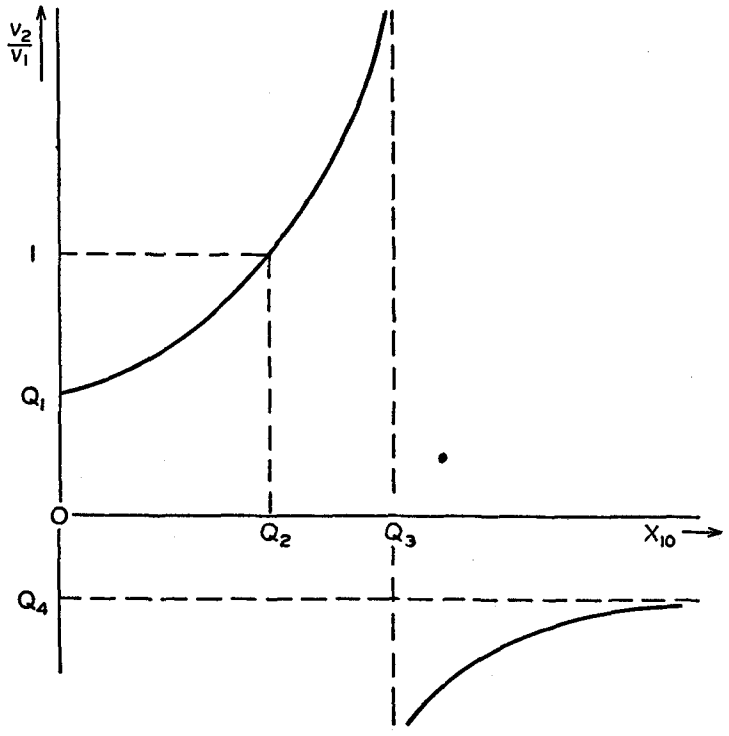

FIGURE 1

partments, each of which is the locus of a particular enzyme. Further, as mentioned before, we shall assume these enzymes to be sufficiently far apart to make diffusion the principal agent of transfer from the site of one step to the site of the other. It was mentioned before that recent electron microscope observations of mitochondria (Palade, *loc. cit.*) show the existence of structures which could well serve as enzymatic loci (Schneider, 1953).

We will investigate the kinetics of such a two-compartment system in which the following reaction occurs:

$$\left.\begin{aligned} &\bar{C}_1+\bar{X}_0 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} X_{11}+\bar{B}_1\,, \\ &X_{12}+\bar{C}_2 \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \bar{B}_2+\bar{X}_{22}\,. \end{aligned}\right\} \tag{16}$$

The first step can occur only in the first compartment because the enzyme catalyzing this step is localized there. The product of this reaction, X_{11}, diffuses out of this compartment, partly into the medium and partly into the second compartment. There, designated as X_{12}, part of it reacts with the localized enzyme of the second step to form $\bar{X}_{22}$ and $\bar{B}_2$ and part also diffuses out into the medium. The effect of the enzymes catalyzing these reactions is expressed through the rate constants, k_i, k_{-i}. As in the uniform system, we shall assume that X_{11} and X_{12} are time dependent variables. For the purpose of this discussion $\bar{C}_1$, $\bar{C}_2$, $\bar{B}_1$, $\bar{B}_2$, $\bar{X}_0$, and $\bar{X}_{22}$ are kept constant. In particular, we set $\bar{w}_i \equiv k_i\bar{C}_i$, $\bar{w}_{-i} = k_{-i}\bar{B}_i$.

In line with previous notation, $G_1(X_{11})$ represents the flow rate per unit volume of X_{11} into the medium, $G_2(X_{11})$ the flow rate of X_{11} per unit volume from compartment 1 into compartment 2, and $G_3(X_{12})$ the flow rate per unit volume of X_{12} out of the second compartment into the medium. Hence the system is described by the following differential equations

$$\left.\begin{aligned} \frac{dX_{11}}{dt} &= \bar{w}_1\bar{X}_0-\bar{w}_{-1}X_{11}-G_1(X_{11})-G_2(X_{11})\,, \\ \frac{dX_{12}}{dt} &= \bar{w}_{-2}\bar{X}_{22}-\bar{w}_2X_{12}+G_2(X_{11})-G_3(X_{12})\,. \end{aligned}\right\} \tag{17}$$

We now set $G_1(X_{11})$ equal to $\lambda_1(X_{11} - X_{10})$, $G_2(X_{11})$ equal to $\lambda_2(X_{11} - X_{12})$, and $G_3(X_{12})$ equal to $\lambda_3(X_{12} - X_{10})$. Since we assume that the volume of each compartment is exactly one-half of the total volume in the corresponding two-step uniform system, the flow coefficients in the two compartments of area A and volume V can therefore be written as $DA/V\Delta l_2$ on the simplifying assumption that the two compartments are identical with respect to permeability and diffusion properties. Furthermore, if we assume that the particles are cylindrical in shape and that the amount diffusing out through the ends is negligible as compared to that diffusing through the sides, Δl_2 can be equated to Δl_1. Hence λ_1 and λ_3 are each separately equal to λ of the uniform system.

Setting (17) equal to zero, the steady-state rate per unit volume of the

first step, $p_1 = \bar{w}_1\bar{X}_0 - \bar{w}_{-1}X_{11}$, can be easily found. The expression for X_{11} is given by

$$X_{11} = \frac{\bar{w}_1\bar{X}_0(\bar{w}_2 + \lambda_2 + \lambda_3) + \lambda_2\bar{w}_{-2}\bar{X}_{22} + X_{10}(\Omega + \lambda_1\bar{w}_2)}{\bar{w}_2(\bar{w}_{-1} + \lambda_1 + \lambda_2) + \bar{w}_{-1}(\lambda_2 + \lambda_3) + \Omega}, \tag{18}$$

where $\Omega = \lambda_1\lambda_3 + \lambda_1\lambda_2 + \lambda_2\lambda_3$. Hence

$$p_1 = \frac{\bar{w}_1\bar{X}_0[\Omega + \bar{w}_2(\lambda_1 + \lambda_2)] - X_{10}\bar{w}_{-1}(\lambda_1\bar{w}_2 + \Omega) - \lambda_2\bar{w}_{-1}\bar{w}_{-2}\bar{X}_{22}}{\Omega + \bar{w}_2(\lambda_1 + \lambda_2 + \bar{w}_{-1}) + \bar{w}_{-1}(\lambda_2 + \lambda_3)}. \tag{19}$$

Similarly, to find $p_2 = \bar{w}_2X_{12} - \bar{w}_{-2}\bar{X}_{22}$, we first determine X_{12}. It is given by

$$X_{12} = \frac{\bar{w}_1\bar{X}_0\lambda_2 + \bar{w}_{-2}\bar{X}_{22}(\bar{w}_{-1} + \lambda_1 + \lambda_2) + X_{10}(\Omega + \lambda_3\bar{w}_{-1})}{\bar{w}_2(\bar{w}_{-1} + \lambda_1 + \lambda_2) + \bar{w}_{-1}(\lambda_2 + \lambda_3) + \Omega}. \tag{20}$$

The expression for p_2 follows immediately. It is

$$p_2 = \frac{\bar{w}_1\bar{w}_2X_0\lambda_2 + \bar{w}_2X_{10}(\Omega + \lambda_3\bar{w}_{-1}) - \bar{w}_{-2}\bar{X}_{22}(\Omega + \bar{w}_{-1}\lambda_2 + \bar{w}_{-1}\lambda_3)}{\Omega + \bar{w}_2(\lambda_1 + \lambda_2 + \bar{w}_{-1}) + \bar{w}_{-1}(\lambda_2 + \lambda_3)}. \tag{21}$$

These two results now enable us to compare the rates of uniform and non-uniform systems. To do this, we must of course compare rates for the whole reaction volume. This means that (19) and (21) must each be multiplied by V, the volume of the reaction compartment, while the corresponding expressions for the uniform system, (7) and (9), must be multiplied by $2V$. Furthermore, some assumption must be made concerning the relative magnitudes of the concentrations of the constant metabolites in the two systems.

As was indicated before, the only difference between the uniform and non-uniform systems is that in the latter the enzymes are concentrated in special regions inside the particle. Therefore there is no reason to assume that the concentrations $\bar{C}_1$, $\bar{C}_2$, $\bar{B}_1$, $\bar{B}_2$, $\bar{X}_0$, and $\bar{X}_2$ are affected by the enzyme localization. The only reasonable effect of this enzyme localization should be on the rate coefficients, k_i and k_{-i}, i.e., *we should expect, as a first approximation, that concentrating the same amount of enzyme in half the volume would double k_i and k_{-i}.* Hence $\bar{w}_i = 2\bar{w}_i$, $\bar{w}_i = 2w_{-i}$.

We can now evaluate the ratio $2Vv_1/Vp_1$, where v_1 is the steady-state rate of the first step in the uniform system and p_1 the corresponding rate in the non-uniform system. We use the simplifying assumption that $\lambda_1 = \lambda_3 = \lambda$. The following expression results:

$$\frac{2v_1}{p_1} = 1 + \frac{w_{-1}(2w_2 + \lambda_1)}{\lambda_2 D_1}\{2(w_1w_2X_0 - w_{-1}w_{-2}X_2) + \lambda(w_1X_0 - w_{-2}X_2) + \lambda X_{10}(w_2 - w_{-1})\}, \tag{22}$$

where

$$D_1 = (\lambda + w_2 + w_{-1})\,[2w_1X_0w_2 + 2w_1X_0\lambda - 2w_{-1}w_{-2}X_2 - 2w_{-1}w_{-2}X_2 - 2w_{-1}\lambda X_{10} + \frac{1}{\lambda_2}(2w_1w_2\lambda X_0 + w_1X_0\lambda^2 - 2w_{-1}\lambda w_2X_{10} - w_{-1}X_{10}\lambda^2)\,]\,. \tag{23}$$

A number of observations can be made with respect to (22):

1. If λ_2 becomes infinite, the ratio of the steady-state rates approaches 1. It should be pointed out that this is not a self-evident result because, even with infinitely rapid diffusion between the two chambers, the non-uniform system differs from the uniform one in that the compartmentalization of the two reaction steps is still preserved.

2. The denominator D_1 is positive as long as p_1, as given in (19), is positive, i.e., as long as the external concentration X_{10} is small enough to permit the reaction to proceed forward. If this condition is met, and if all forward coefficients, w_1X_0, w_2, are greater than any of the reverse coefficients, w_{-1}, w_2X_2, then the ratio of the steady-state rates is always greater than 1.

3. If λ, the flow coefficient to the medium, becomes very small, the ratio is always greater than 1.

Since, in general, the forward coefficients do tend to be greater than the reverse ones, and since the flow coefficient is probably not too significant, we can conclude that the rate of the uniform system of the first step will tend to be greater than the corresponding non-uniform rate.

A similar calculation can be carried through which compares the steady-state rates of the second step, i.e., $2Vv_2/Vp_2$. This ratio is given by

$$\frac{2\,v_2}{p_2} = 1 + \frac{w_2\,(2w_{-1} + \lambda)}{\lambda_2 D_2}\,\{2\,(w_1w_2X_0 - w_{-1}w_{-2}X_2) + \lambda\,(w_1X_0 - w_{-2}X_2) + \lambda X_{10}\,(w_2 - w_{-1})\,\}, \tag{24}$$

where

$$D_2 = (\lambda + w_2 + w_{-1})\left[2w_1w_2X_0 + 2\lambda w_2X_{10} - 2w_{-2}X_2\,(w_{-1} + \lambda) + \frac{1}{\lambda_2}(2\lambda w_2w_{-1}X_{10} + w_2X_{10}\lambda^2 - 2w_{-1}\lambda w_{-2}X_{22} - \lambda^2w_{-2}X_{22})\right]. \tag{25}$$

We can conclude from a consideration of (24) that

1. the ratio approaches 1 for very large λ_2,
2. the ratio is always greater than 1 for small λ, and
3. if p_2, as given in (21), is positive, i.e., X_{10} bounded from below, then D_2 is positive. If this condition is met, and if the forward coefficients are again greater than the reverse ones, the ratio is greater than 1.

These conclusions, as can be seen, are very similar to the ones in the case

of the first step. We can therefore conclude that under certain assumptions concerning the order of magnitude of the reaction coefficients and flow coefficients, *the steady-state rates of the uniform system will, in general, tend to be greater than of the non-uniform system.*

It may be rewarding to examine this conclusion a little more closely.

There are two factors involved in the difference between uniform and non-uniform systems. One is that in the non-uniform system we posulate a finite flow coefficient; in the uniform system it becomes infinite. The second factor is the effect of compression on the chemical reaction. By decreasing the volume in which a given particular reaction takes place, and hence by increasing the enzyme concentration, the rate per unit volume is accelerated. These two factors operate in opposing directions. It is the particular resolution of these two conflicting factors which determines the relative magnitudes of the steady-state rates in the uniform and non-uniform systems. Since we have shown that in general the uniform rate will tend to be larger than the non-uniform one, we can conclude that the factor of diffusion is more significant than the factor involving the compression of the reacting enzymes into a smaller volume.

That this explanation is correct can be seen immediately from the following considerations.

The steady-state rate of the general reaction system (1) can be written (Hearon, *loc. cit.*)

$$v = \frac{X_0 \prod_1^n \nu_j N_j C_j - X_n \prod_1^n \nu_{-j} M_j B_j}{V\{\nu_2\nu_3 \ldots \nu_n N_2 \ldots N_n C_2 \ldots C_n + \ldots + \nu_{-1}\nu_{-2} \ldots \nu_{-(n-1)} M_1 \ldots M_{n-1} B_1 \ldots B_{n-1}\}} \tag{26}$$

where

$$k_j = \nu_j E_j = \frac{\nu_j N_j}{V}, \qquad k_{-j} = \nu_{-j} E_{-j} = \nu_{-j} \frac{M_j}{V};$$

V represents here the volume of the particle, and N_j and M_j the total amounts of enzyme available.

The total steady-state rate for the whole volume is then given by vV and is independent of the volume of the reaction system. Since the flow coefficient λ is also inversely related to the volume V, it can be seen upon inspection of (7) and (9), for example, that its introduction into the rate expression does not alter this conclusion.

Since a decrease in the reaction volume does not increase the rate, the only effect of compartmentalization is to decrease the steady-state rate because of the finite flow coefficients.

At this point we note that the above results follow from a particular model of the reaction system, a model in which the essential acts, collisions between molecules, are assumed on the average to occur identically in each volume element. This is equivalent to the assumption that the reaction system is essentially homogeneous. This assumption is fairly adequate where the surfaces on which the reactions occur are relatively small as compared to the distances between molecules. On the other hand, when dealing with localized surface catalyzed systems it becomes less certain that this homogeneity actually holds.

A different model, representing the non-homogeneous case, would be that of catalytic surfaces immersed in solution in which reactants and products diffuse to and from the active sites. Here the volume elements do not have the same properties. Diffusion occurs only in a certain region, free from chemical reaction; the chemical reaction occurs in a special area, on the catalytic surfaces.

As the simplest example of this model, consider two surfaces, one at $x = 0$, the other at $x = l$. A substance A present in large quantities in the solution between 0 and l forms B on the surface at $x = 0$ at a rate k_1A which we take to be constant. Substance B diffuses then to $x = l$ where it forms irreversibly a substance C at a rate k_3. We further assume that B can also re-form A at the first surface.

In the steady state the problem becomes mathematically simply one of evaluating the one-dimensional Laplace equation with the boundary conditions

$$\left.\begin{aligned} -D\left(\frac{dB}{dx}\right)_0 &= k_1A - k_2B(0)\ , \\ -D\left(\frac{dB}{dx}\right)_l &= k_3B(l)\ . \end{aligned}\right\} \tag{27}$$

The solution is completely straightforward (Appendix IB). The rate of production of C is given by $k_3B(l)$, and hence equals

$$\frac{Dk_1k_3A}{Dk_3 + Dk_2 + k_2k_3l}. \tag{28}$$

We can see that this rate is inversely dependent on l. As expected, the greater the distance between the catalytic surfaces, the slower the rate. Further, if the reaction is completely irreversible, i.e., k_2 is zero, the steady-state rate is independent of the distance.

Therefore in so far as the enzyme reactions inside of particles can be represented by such arrangements of surfaces, the distances between the enzymes become a significant factor in influencing the steady-state rate.

One way of looking at this effect is to say that the distance between enzymes determines the "necessary" concentration of the substrate. In the above example, suppose we have two identical enzyme systems (two surfaces each), differing only in the distance between the surfaces. To achieve the same rate, different concentrations of A are necessary in the two cases. If l_1 and l_2 represent the respective distances, and A_1 and A_2 the respective necessary concentrations of A, we find, from (28),

$$A_1 = A_2 \frac{D(k_3 + k_2) + k_2 k_3 l_1}{D(k_3 + k_2) + k_2 k_3 l_2}. \tag{29}$$

If the diffusion coefficient D of the substance B is large compared to $(k_2 k_3 l_i)/(k_3 + k_2)$ this ratio becomes 1. But if diffusion is not so rapid, the necessary concentration A_1 can decrease to $A_2 l_1/l_2$.

We can also compare this surface reaction with the corresponding homogeneous reaction. It can be shown very easily that if the distance l between the surfaces becomes small enough the surface rate will be larger than the homogeneous rate.

In the previous example, let us assume that the number of active sites on a unit surface of that system corresponds to the enzyme concentration of a unit volume. Hence the steady-state rate, per unit volume of the homogeneous system, is given by

$$\frac{k_1 k_3 A}{k_2 + k_3}. \tag{30}$$

This is of course identical with (28) when D becomes infinite. Equation (30) must now be compared with the corresponding surface rate per unit volume, which in this case is simply $k_3 B(l)/l$. It can be seen that the smaller l, the greater the surface rate. In particular, the two rates are equal when l equals

$$\frac{-D(k_2 + k_3) + \sqrt{[D^2(k_2 + k_3)^2 + 4Dk_2 k_3(k_2 + k_3)]}}{2k_2 k_3}. \tag{31}$$

For smaller l, the surface rate is larger than the volume rate. When D becomes very large, (31) approaches 1, as expected.

It can therefore be concluded that if we view the reactions from the standpoint of surface systems, compression of volume does result in increase of rate. And it can further be assumed that for localized systems such as mitochondria, in which the dimensions between enzymes are fairly small, the surface model is a better approximation than the volume model.

II. Concentration of intermediates. Another criterion by which we can compare uniform and non-uniform systems refers to the total amount of intermediates stored in the reaction system in the steady state. This can be of importance biologically for the following reasons:

1. At certain times intermediates may suddenly seep from the reaction system. Complete drainage makes it difficult to reestablish the desired steady-state rate. This seepage could be due, for example, to injury of the system, or to the necessity of adjusting to an unusual temporary environment.

2. Some of the metabolites reacting with the intermediates can also be used in other reactions. Hence competition exists for these metabolites, which will be greatly affected by the amounts of competing intermediates This follows from the law of mass action.

Therefore for both of these reasons it seems useful for the metabolic system to have stores of intermediates available.

Now consider the diffusing uniform system. The concentration of intermediate is given by (6). When λ becomes zero (6) becomes the corresponding concentration $\bar{X}_1$ for the non-diffusing system. The ratio of the two expressions is given by

$$\frac{X_1}{\bar{X}_1} = 1 + \frac{\lambda\,[X_{10}\,(w_2+w_{-1}) - w_1X_0 - w_{-2}X_2]}{(w_1X_0+w_{-2}X_2)\,(\lambda+w_2+w_{-1})}. \tag{32}$$

Clearly, if X_{10} is small, $\bar{X}_1$, the concentration in the closed system, is larger than X_1; for X_{10} greater than $(w_1X_0 + w_{-2}X_2)/(w_2 + w_{-1})$, X_1 is larger. Hence, as in the case of the steady-state rate, the external concentration becomes a mechanism for adjustment of the intermediate concentration.

We can similarly compare the concentration of intermediates in the uniform and non-uniform systems. To make this comparison meaningful we have to compare total amounts of intermediate, which is given in the two-step case by the ratio $2X_1/(X_{11} + X_{12})$. This ratio can be computed very easily from (6), (18), and (20):

$$\frac{2X_1}{X_{11}+X_{12}} = 1 - \frac{(w_2-w_{-1})}{\lambda_2 D_3} \times [2\,(w_1w_2X_0 - w_{-1}w_{-2}X_2) + \lambda X_{10}\,(w_2-w_{-1}) - \lambda\,(w_{-2}X_2 - w_1X_0)\,], \tag{33}$$

where

$$D_3 = (\lambda+w_2+w_{-1})\left\{2w_1X_0 + 2w_{-2}X_2 + 2\lambda X_{10} + \frac{1}{\lambda_2} \times [w_1X_0\,(2w_2+\lambda) + w_{-2}X_2\,(2w_{-1}+\lambda) + \lambda X_{10}\,(w_2+\lambda+w_{-1})]\right\}.$$

A number of observations can be made with respect to (33):

1. If λ_2 becomes very large, the ratio approaches 1, as expected.
2. If λ is very small and w_2 greater than w_1, the ratio is less than 1.
3. If w_{-1} is greater than w_2 and λ is negligible, the ratio is greater than 1.

Since on the basis of experimental evidence λ can be considered quite small, and the forward coefficients are generally considerably larger than the backward coefficients, we can conclude that the total amount of intermediate present in the non-uniform system will be larger than in the uniform system.

III. Access of substrates to enzymes. One of the factors listed by Danielli (*loc. cit.*) as being significant in control of enzyme systems is the access of substrate to enzyme. There are two aspects to this:

1. It is advantageous to the cell that certain reactions proceed well. The cellular structures would then be so designed as to channel the substrate toward the desired enzymes.

2. It is advantageous to the cell that certain reactions be suppressed. The cellular structures would then be so designed as to prevent access of substrate to the particular enzymes.

It is clear on the basis of the previous discussion that a structure such as a mitochondrion can be very useful in focusing substrate flow toward the desirable enzymes and eliminating loss in seepage or undesirable side reactions. As a matter of fact, any system of membranes relatively impermeable to the substrate and so arranged as to permit flow only toward the particular enzyme would accomplish this result.

Another method whereby access of substrate to a particular enzyme is enhanced and access to an undesired side reaction prevented is spatial localization with respect to the substrate source. Consider the following simple situation. A substrate S is homogeneously dissolved in a medium. It can react with C_1 to form p_1, or with C_2 to form p_2. The rate of the first step, q_1, is k_1C_1S; that of the second step, q_2, is k_2C_2S. Hence

$$\frac{q_1}{q_2} = \frac{k_1C_1}{k_2C_2}. \tag{34}$$

As seen from (34), in the case where spatial localization does not enter, the only factor determining the amount of substrate flowing to the desired goal, p_1, and the undesired goal, p_2, is the ratio of the concentrations of C_1 and C_2.

Now consider the following localized system. As shown in Figure 2, the source of S is at A. It diffuses in from the source along the X-axis into a

compartment where only C_1 is present, or, if preferred, where the enzyme catalyzing the first reaction is localized. Hence, in this compartment,

$$S_1 + C_1 \rightarrow p_1 ,$$

where S_1 is the concentration of S in that region. Whatever remains of S_1 diffuses further toward B where the second enzyme is localized. Here p_2 is produced according to the reaction

$$S_2 + C_2 \rightarrow p_2 ,$$

where S_2 is the substrate concentration in the second region. It is clear that

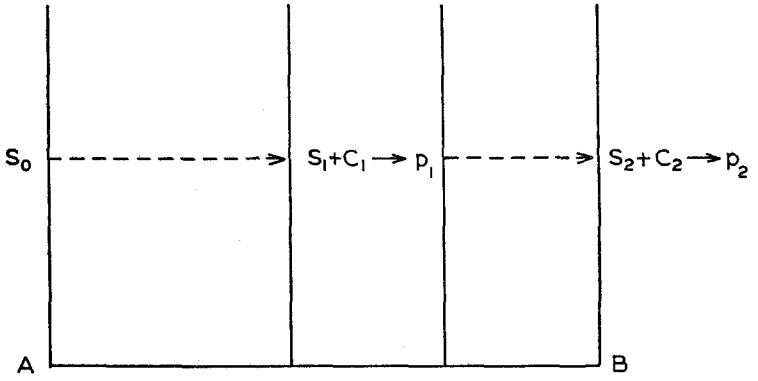

FIGURE 2

such a spatial arrangement greatly enhances the accessibility of S to C_1 and reduces it to C_2. The differential equations of this system are

$$\left.\begin{aligned} \frac{dS_1}{dt} &= \lambda_1 (S_0 - S_1) - k_1 S_1 C_1 - \lambda_2 (S_1 - S_2) , \\ \frac{dS_2}{dt} &= \lambda_2 (S_1 - S_2) - k_2 S_2 C_2 . \end{aligned}\right\} \tag{35}$$

The ratio of the steady-state rates, $\bar{q}_1 = k_1 C_1 S_1$, $\bar{q}_2 = k_2 C_2 S_2$, is found by solving for S_1 and S_2 from the steady-state expressions corresponding to (35). It follows that

$$\frac{\bar{q}_1}{\bar{q}_2} = \frac{k_1 C_1}{k_2 C_2}\left(1 + \frac{k_2 C_2}{\lambda_2}\right). \tag{36}$$

To compare (36) with (34), assume that a certain ratio $(q_1/q_2)^*$ is desired. To make this feasible in the uniform system, a concentration

$$C_1 = \frac{k_2 C_2}{k_1}\left(\frac{q_1}{q_2}\right)^* \tag{37}$$

is required. Clearly, the larger C_2, the greater C_1 must be. But in the localized system, using (36), C_1 is required to be

$$C_1 = \frac{k_2 C_2}{k_1} \left(\frac{q_1}{q_2}\right)^* \left(\frac{1}{1 + \frac{k_2 C_2}{\lambda_2}}\right). \tag{38}$$

As expected, the smaller λ_2, the smaller the required C_1. Further, as C_2 increases, C_1 does not become arbitrarily large, but reaches the asymptotic value of

$$\left(\frac{q_1}{q_2}\right)^* \frac{\lambda_2}{k_1}.$$

Enzyme localization, as described, is therefore a simple and effective mechanism for controlling reaction systems, enhancing certain reactions, and suppressing others. Such a mechanism could conceivably be involved in the well known transformation principle (Braun, 1953) or the alteration in bacterial synthesis upon viral infection (Epstein, 1953). In both cases, outside agents consisting largely of desoxy-ribose-nucleic acid (DNA) shift the synthetic pathways into new channels. It is suggested that DNA might be able to accomplish this, perhaps, by locating itself near the sources of energy and/or substrates being competed for, thus suppressing the normal enzyme pathways.

We have mentioned so far two structural features involved in substrate accessibility, namely, permeability features (membranes, etc.), which prevent flow in undesired directions, and enzyme localization of the kind described in the model above. There is another way in which enzyme localization could conceivably effect access of substrate to enzyme. It is well known that the addition of a colloid to a solution increases the solution viscosity. A particular expression for this viscosity, due to Einstein, is the well known expression

$$\eta = \eta_0 (1 + 2.5\phi), \tag{39}$$

where η is the coefficient of viscosity of the suspension, η_0 that of the dispersion medium, and ϕ the relative volume concentration of the suspension (Gortner and Gortner, 1950). Since the diffusion coefficient is generally considered to be inversely related to the viscosity of the suspension in which diffusion takes place, it can therefore be concluded that enzyme localization could give rise to a considerable decrease in the rate of diffusion of substrate in the localization region.

IV. Oscillations and overshoot. Although most of the work done so far on cellular metabolic systems has dealt with the steady state, nevertheless a

good number of investigations have also been made of the time dependent behavior of these systems. Most attention has been paid here to the possibility of periodic behavior, and to the possibility of overshoot.

A. Periodic behavior. Burton (1939), in his pioneering article, indicated interest in possible periodic behavior of open systems, but did not actually study it. A detailed investigation of the periodic behavior of autocatalytic systems was made by M. Moore (1949). She showed that those systems possess periodicities which are physically feasible. Hearon (1953) studied the possibility of periodicities in open and closed linear systems and came to the conclusions that, under certain general conditions, periodicities are not possible for them.

We will attempt here to carry this investigation somewhat further, emphasizing in particular the effect of localization on possible periodicity. Unfortunately no general, easily utilizable criterion is known to the author by which periodic behavior can be definitely established.

The following procedure will be followed. In all cases dealing with various nonlinear systems, we shall assume that the system fluctuates only slightly from a steady state and hence we will be able to transform the original nonlinear differential equations into linear differential equations. These linear equations involve the amplitudes of fluctuations from the steady state.

The solution of this set of linear equations will be of the form $\Sigma u_i \exp(-\lambda_i t)$ and the λ_i will be determined as the roots of the auxiliary equation of the form

$$\lambda^n + a_1\lambda^{n-1} + a_2\lambda^{n-2} + \ldots + a_n = 0 \,. \tag{40}$$

The solution will be oscillatory if any of the λ_i's are complex; our problem therefore becomes one of determining the existence of complex roots of (40) without having to solve it explicitly. Unfortunately, the author is aware of only a sufficient condition for the existence of complex roots (Turnbull, 1944). If $a_1^2 \leq 2a_2$ in (40), complex roots exist. Of course, the condition may not be satisfied and yet complex roots will occur. This condition is particularly useful to us, because, given the determinant of coefficients from which (40) is derived, we can easily determine the two coefficients of λ^{n-1} and λ^{n-2}, a_1, and a_2.

Consider a set of n differential equations

$$\frac{dy_i}{dt} = \sum_{j=1}^{n} a_{ij}y_j \,, \qquad i = 1, 2 \ldots n. \tag{41}$$

On the assumption that $y_i = \Sigma u_{ij} \exp(-\lambda t)$, (41) becomes

$$0 = \sum_{j=1}^{n} y_j (a_{ij} + \lambda \delta_{ij}) , \quad i = 1, 2, \ldots n, \quad (42)$$

where δ_{ij} is Kronecker's delta. When the determinant of the coefficients of (42) is expanded, the auxiliary equation (40) results. The coefficient a_1 of λ^{n-1} is given simply by

$$\sum_{i=1}^{n} a_{ii} .$$

The coefficient a_2 of λ^{n-2} consists of two series. The first series is

$$\frac{1}{2} \sum_{i=j}^{n} a_{ii} a_{jj} \overset{i}{-} \sum_{1}^{n} a_{ii}^2$$

and the second

$$-\frac{1}{2} \sum_{i,\, j=1}^{n} a_{ij} a_{ji} , \qquad i \neq j$$

i.e., the sum of all products of all diagonal terms subtracted from the first series. It is clear that $(\Sigma a_{ii})^2 > \Sigma a_{ii} a_{jj} - 2\Sigma a_{ii}^2$. Hence a_1^2 can only be less than $2a_2$ if the second series of a_2 is negative, thus canceling the minus sign in front. This can only be true if at least one a_{ij} has the opposite sign of a_{ji}. If this condition is not satisfied, we have clear proof that the sufficient condition is not satisfied.

This criterion can now be applied to various systems. The following do not meet the above requirements:

1. the linear uniform system,

$$X_{i-1} + C_i \rightleftarrows B_i + X_i ,$$

as expected from Hearon's investigation,

2. the same system as in 1., except that it is made nonlinear by treating the C_i's and B_i's as functions of time,

3. an open enzyme system of the Michaelis-Menton type with the modification that its second step be reversible, e.g.,

$$E_i + S_i \underset{k_{-i}}{\overset{k_i}{\rightleftarrows}} X_i \qquad i = 1, 2, \ldots n$$

$$X_i \underset{\sigma_{-i}}{\overset{\sigma_i}{\rightleftarrows}} S_{i+1} + E_i ,$$

(S_i is the ith substrate, X_i the ith enzyme-substrate complex), and

4. the non-uniform system, analogous to 1. and 2. above.

From the four cases above it can be assumed that periodicity is not a widespread property of simple kinetic systems.

The following systems do satisfy, or can satisfy, the sufficient condition for periodicity:

1. The closed reversible enzyme system. This differs from 3. in that the equation of conservation of mass is used. If K is the total amount of material present in the system,

$$K = \sum_{1}^{n} (S_i + X_i) + S_{n+1} . \tag{43}$$

Setting $\alpha_i = X_i - \bar{X}_i$ and $\beta_i = S_i - \bar{S}_i$, where $\bar{X}_i$ and $\bar{S}_i$ are steady-state concentrations, and using (43) to eliminate S_1, the determinant of coefficients of the α_i's and β_i's now has one diagonal pair with opposite signs (Appendix IVA), namely,

$$\left.\begin{aligned} a_{1(n+1)} &= -(k_1 - \sigma_{-1})(E_{o1} - X_1) , \\ a_{(n+1),1} &= \sigma_1 + \sigma_{-1}\bar{S}_2 . \end{aligned}\right\}$$

Hence if $k_1 > \sigma_{-1}$, the barest possibility of oscillation exists. Clearly, the greater n, the number of steps in the system, the smaller the possibility that this one term can outweigh the other negative terms.

But it can be easily shown that oscillations are *not* possible in the most favorable case, i.e., $n = 1$. The characteristic equation of this system becomes (Appendix IVB)

$$\begin{aligned} \lambda^2 - \lambda\{k_1(\bar{E}_1 + \bar{S}_1) + \sigma_1 + k_{-1} + \sigma_{-1}(\bar{E}_1 + \bar{S}_2)\} \\ + \bar{E}_1\{\sigma_{-1}k_1(\bar{E}_1 + \bar{S}_1 + \bar{S}_2) + \sigma_{-1}k_{-1} + \sigma_1 k_1\} = 0 . \end{aligned} \tag{44}$$

Oscillations cannot occur if $b^2 - 4ac \geq 0$, where b is the coefficient of λ, $a = 1$, and c is the constant term in (44). By simple rearrangement of the coefficients of (44) it is easily shown (Appendix IVB) that $b^2 - 4ac$ is *always* positive.

We can therefore conclude that closed reversible systems of the type described will *not* oscillate.

2. It was shown by Moore (*loc. cit.*) that autocatalytic systems show oscillatory behavior. She investigated n-step systems of the type described here as diffusible uniform systems. We shall therefore investigate similar non-uniform systems.

Consider n linearly arranged compartments, as shown in Figure 3. The metabolites are designated A_{ij}, where A_{kl} is the concentration of the kth metabolite in the lth compartment. In all cases A_{jj} is produced in the jth

compartment from $A_{j-1,j}$ autocatalytically, i.e., at the rate $k_j A_{jj} A_{j-1,j}$. The metabolite A_{jj} diffuses out of the compartment into the medium at the rate $\sigma_{jj} A_{jj}$ and into the next $(j+1)$th compartment at the rate $r_j(A_{jj} - A_{j,j+1})$. The only exception is in the first compartment, where A_{11} is produced at the rate KA_{11}. The differential equations describing this system are

$$\left.\begin{aligned} \frac{dA_{j,j+1}}{dt} &= r_j(A_{jj} - A_{j,j+1}) - k_j A_{j,j+1} A_{j+1,j+1} - \sigma_{j,j+1} A_{j,j+1} \\ \frac{dA_{j+1,j+1}}{dt} &= k_j A_{j,j+1} A_{j+1,j+1} - r_{j+1}(A_{j+1,j+1} - A_{j+1,j+2}) \\ &\quad - \sigma_{j+1,j+1} A_{j+1,j+1} . \end{aligned}\right\} \quad (45)$$

The solution of (45) is straightforward and the determinant of coefficients easily ascertained (Appendix IVC). If $\bar{A}_{ij}$ represents the steady-state

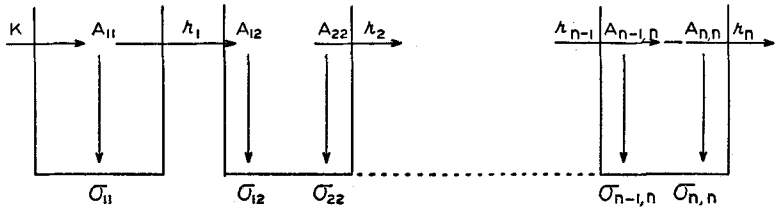

FIGURE 3

value of A_{ij}, we can solve for the two coefficients of λ^{n-1} and λ^{n-2}. They are

$$\left.\begin{aligned} a_1 &= \sum_{j=1}^{2(n-1)} Q_j , \quad Q_1 = \frac{r_1 \bar{A}_{12}}{\bar{A}_{11}} , \quad Q_2 = \frac{r_1 \bar{A}_{11}}{\bar{A}_{12}} , \quad Q_3 = \frac{r_2 \bar{A}_{23}}{\bar{A}_{22}} , \quad \cdots \\ a_2 &= \frac{1}{2} \sum_{i,j=1}^{2(n-1)} Q_i Q_j - \sum_{1}^{n-1} r_j^2 + \sum_{1}^{n-1} k_j^2 \bar{A}_{j,j+1} \bar{A}_{j+1,j+1} . \end{aligned}\right\} \quad (46)$$

Hence

$$a_1^2 - 2a_2 = \sum_{1}^{2(n-1)} Q_j^2 + 2\sum_{1}^{n-1} r_j^2 - 2\sum_{1}^{n-1} k_j^2 \bar{A}_{j,j+1} \bar{A}_{j+1,j+1} .$$

As can be seen from (46), the sufficiency condition for periodicity is not always satisfied. Since in the case of uniform systems studied by Moore periodicity always occurs, we can immediately conclude that compartmentalization militates against periodicity.

It can also be seen from (46) (Appendix IVC) that the sufficiency condition is satisfied if

$$r_j^2 \leq \frac{2k_j^2 [\bar{A}^3_{j,j+1}\bar{A}_{j+1,j+1}\bar{A}^2_{jj}]}{2\bar{A}^2_{jj}\bar{A}^2_{j,j+1} + \bar{A}^4_{j,j+1} + \bar{A}^4_{j,j}} . \tag{47}$$

Hence the flow coefficient r_j must be bounded from above. It should be pointed out here that this non-uniform autocatalytic system reduces to the uniform system by setting $A_{j,j+1}$ equal to A_{jj}, which, using (45), eliminates all terms involving r_j.

B. Overshoot. Burton (*loc. cit.*) discussed in some detail the biological significance of overshoot phenomena. Some of the examples cited by him as evincing this property are: positive overpotential in nerve, oxygen consumption in bacteria and Arbacia eggs, and the so-called "vagus escape." This same property of open systems was also investigated by Denbigh, Hicks, and Page (1948). Overshoot is defined as occurring when a system in moving toward a steady state from one side crosses the steady-state value and takes on values on the other side of the steady state.

Mathematically, it means the following. Let the displacement of the system from the steady state be given by $\Sigma u_i \exp(-\gamma_i t)$. Overshoot occurs, then, whenever this series changes sign. In general, we can see that the larger the number of terms in this series, the greater the possibility that such a reversal can occur. In particular, since a non-uniform system either always has $2n - 1$ or $2n$ terms, the non-uniform system has a larger number of parameters which can be adjusted so as to make overshoot possible for a corresponding uniform system of n terms.

As the simplest possible example consider a 2-step system with uniform enzyme distribution. If we set $\chi = X_1 - \bar{X}_1$, where $\bar{X}_1$ is the steady-state value, the following differential equation results

$$\frac{d\chi}{dt} = -(w_{-1} + w_2)\chi .$$

The solution is $\chi = \nu e^{-(w_{-1}+w_2)t}$, and overshoot is clearly not possible. In the equivalent non-uniform system,

$$C_1 + X_0 \leftrightarrow X_{11} + B_1 ,$$

$$X_{12} + C_2 \leftrightarrow X_2 + B_2 ,$$

we set $\chi_1 = X_{11} - \bar{X}_{11}$, $\chi_2 = X_{12} - \bar{X}_{12}$, and from the resulting differential equations find (Appendix IVD)

$$\left.\begin{aligned} \chi_1 &= \mu_1 e^{-\gamma_1 t} + \mu_2 e^{-\gamma_2 t} , \\ \chi_2 &= \beta_1 e^{-\gamma_1 t} + \beta_2 e^{-\gamma_2 t} , \end{aligned}\right\} \tag{48}$$

where

$$\gamma_{1,2} = \frac{2\lambda + w_2 + w_{-1} \pm \sqrt{4\lambda^2 + (w_2 - w_{-1})^2}}{2}. \tag{49}$$

If we set χ_{10} equal to $\chi_1(0)$ and χ_{20} equal to $\chi_2(0)$, we can then solve for the coefficients in (48). In particular, for χ_1 we find

$$\left.\begin{aligned} \mu_1 &= \frac{\chi_{10}(\lambda + w_{-1} - \gamma_2) - \lambda\chi_{20}}{\gamma_1 - \gamma_2}, \\ \mu_2 &= \frac{\lambda\chi_{20} - \chi_{10}(\lambda + w_{-1} - \gamma_1)}{\gamma_1 - \gamma_2}. \end{aligned}\right\} \tag{50}$$

In order to have overshoot χ_1 must be zero for some finite time t^*. According to (48) this requires that

$$-\frac{\mu_1}{\mu_2} = \exp\ (\gamma_1 - \gamma_2)\, t^*.$$

Hence if $\gamma_1 > \gamma_2$, $-(\mu_1)/(\mu_2) > 1$. The latter requirement, considering (49) and (50), is equivalent to (Appendix IVD)

$$w_2 - w_{-1} - 2\lambda\frac{\chi_{20}}{\chi_{10}} > [4\lambda^2 + (w_2 - w_{-1})^2]^{1/2}. \tag{51}$$

Equation (51) is feasible only if $(\chi_{20})/(\chi_{10}) < 0$. Following the argument of Burton in his article mentioned above, we now examine the conditions for which $(\chi_{20})/(\chi_{10}) < 0$ holds. In particular, we require that χ_{10} and χ_{20} represent *differences* in steady-state values of X_{11} and X_{12}. These steady-state values are given by (18) and (20), with the simplification of setting $\lambda_1 = \lambda_3 = 0$.

Inspection of these equations discloses that the inequality $(\chi_{20})/(\chi_{10}) < 0$ *cannot* be satisfied for any variations in $\bar{X}_0$, $\bar{X}_2$, or in any of the chemical reaction constants $\bar{w}_i$, $\bar{w}_{-i}$. It is, however, satisfied by a change in the flow coefficient λ. In particular, if λ goes to $\lambda + \Delta\lambda$, χ_{10} becomes $\bar{w}_2\Delta\lambda(\bar{w}_{-1}\bar{w}_{-2}\bar{X}_2 - \bar{w}_1\bar{w}_2\bar{X}_0)$ and χ_{20} becomes $-\bar{w}_{-1}\Delta\lambda(\bar{w}_{-1}\bar{w}_{-2}\bar{X}_2 - \bar{w}_1\bar{w}_2\bar{X}_0)$. This is completely different from the situation found by Burton for a uniform system. There overshoot was only possible for a variation in the reaction constants, and impossible for a variation in the diffusion coefficients. We can therefore conclude that non-uniformity in the kinetic system alters the requirements and conditions of overshoot.

We can now summarize the results of this last section: periodicities are not common characteristics in simple kinetic systems, at least as determined by the sufficiency condition. Their occurrence is reduced in non-uniform systems as compared to uniform ones. In particular, the flow

coefficient λ, unique to the non-uniform system, has a sign which militates against periodicities.

Owing to the larger number of terms in the solution of the equations of non-uniform systems, more parameters can be adjusted to make overshoot possible. In comparing a 2-step non-uniform system with a similar uniform one as described by Burton, we find that the conditions for overshoot are altered as a result of the non-uniformity.

V. Multiple steady states. Another property which is of biological interest is that of multiple steady states. We mean by this the ability of a kinetic system to possess more than one stable steady state and to move from one to the other if sufficiently displaced. Most steady-state systems discussed in the literature (Burton, *loc. cit.*; Denbigh, Hicks, and Page, *loc. cit.*; Bertalanffy, 1950) show only one stable steady state, and for these systems the final state is independent of the initial starting point and only dependent on the fixed parameters of the system. This condition has been described as "equifinality," as was pointed out in a previous note (Bierman, 1954); this is not a general property of steady-state systems, since, clearly, systems possessing multiple steady states will move toward one rather than another of the possible steady states dependent on the initial starting point upon displacement.

This property is not unique, but occurs frequently in nonlinear systems (Minorski, 1947; Rashevsky, *loc. cit.*). It is also of biological interest because shifts from one level to another do occur and are of decisive importance, e.g., in genetics (mutations), adaptations, or embryology. Therefore it seems useful to inquire what kinds of systems can give rise to multiple steady states and how structure relates to this property, if at all.

We have used the graphical method in this investigation, because it is the simplest. For example, given two variables with respect to time, $x(t)$ and $y(t)$, whose first-order differential equations are nonlinear functions $\dot{x}(t) = P_1(x, y)$, $\dot{y}(t) = P_2(x, y)$, $P_1(x, y) = 0$, and $P_2(x, y) = 0$ are then plotted in the x, y plane. The intersections of these two curves represent steady states. The stability of these steady states must then be determined by going back to the original differential equations and noticing the effect of displacement from the steady state on the variables x and y.

Clearly this method is really only applicable to two variables. For three variables the method can be extended to a three-dimensional model, but this becomes complicated already. We have therefore limited ourselves here to considerations of two variables.

The following systems were investigated and were shown *not* to possess multiple steady states:

1. a 2-step linear uniform system,

2. a 2-step uniform system made nonlinear by letting B_1 become a variable of time,

3. the corresponding nonlinear non-uniform system,

4. a reversible 2-step enzyme system,

5. a 2-step system where A forms B via the Langmuir adsorption isotherm $k_1A/1 + \sigma_1A$, and is similarly re-formed from B at the rate $k_2B/1 + \sigma_2B$, and

6. a 2-step autocatalytic system. This was treated by Denbigh, Hicks, and Page (*loc. cit.*) and was shown to possess one stable and one unstable steady state.

From the list above it is apparent that the simpler nonlinear kinetic systems, both uniform and non-uniform, do not possess multiple steady states. It was possible, though, to find one 2-step system possessing two stable steady states; this system involves autocatalysis as well as surface reactions. It can be described as follows:

Let x be produced via a Langmuir adsorption isotherm at the rate $k_1x/1 + \sigma_1x$. It then can either diffuse out of the system at the rate k_3x or react with another metabolite y to form a complex xy. This complex goes over to y via a surface reaction described by $k_2xy/1 + \sigma_2xy$. This is also an autocatalytic reaction by y. The metabolite y can also diffuse out of the system at the rate k_4y.

This system, which possesses multiple stable steady states, is then described by the following nonlinear differential equations

$$\left.\begin{aligned} \frac{dx}{dt} &= \frac{k_1x}{1+\sigma_1x} - \frac{k_2xy}{1+\sigma_2xy} - k_3x\,, \\ \frac{dy}{dt} &= \frac{k_2xy}{1+\sigma_2xy} - k_4y\,. \end{aligned}\right\} \tag{52}$$

Before proceeding with an analysis of (52), it is of interest to point out, first of all, that neither a pure, autocatalytic system nor a purely surface reaction system alone gives rise to multiple steady states. A system that combines both those features, however, can do so. Secondly, both surface kinetics and autocatalysis are significant and characteristic features of biological reaction kinetics, and are moving more and more into the forefront of investigation in such areas as virology, genetics, and enzymology. Thirdly, surface reaction systems are generally indicative of enzyme localization and hence associated with what we called non-uniform systems.

The graphical analysis of this autocatalytic and surface system is given in Figures 4, 5, and 6. In each case the various intersections of the two curves represent the steady-state positions in the x, y plane. The directions of the arrows indicate the stability of these steady states. If the arrows lead back to the steady state, it is stable, i.e., any displacement gives rise to compensatory processes. If the arrows lead away from the steady state,

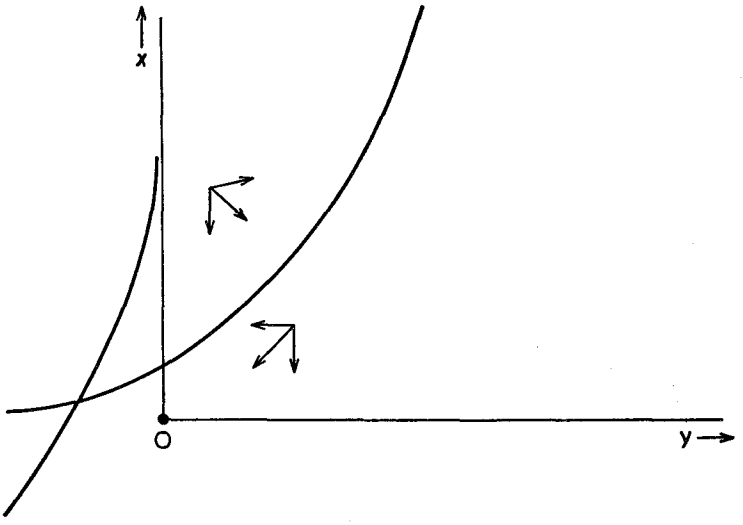

FIGURE 4. The case: $k_1 < k_3$

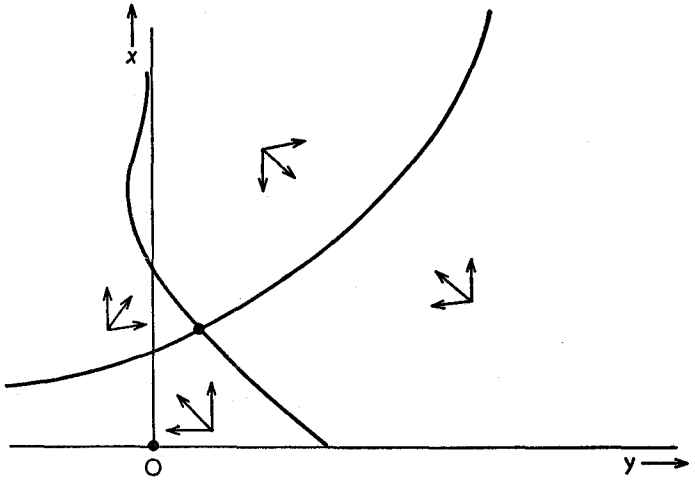

FIGURE 5. The case: $k_3 < k_1 < k_3 + k_2(\sigma_1)/(\sigma_2)$

it is unstable. For details, see Appendix VA. It should also be mentioned that one steady state of this system is given by $x = y = 0$, which is not given as an intersection but shown as a point in the figures.

Three possibilities must now be considered:

1. Case: $k_1 < k_3$. This system is represented in Figure 4 and described in Appendix VA. As can be seen from the graph, there is only one stable

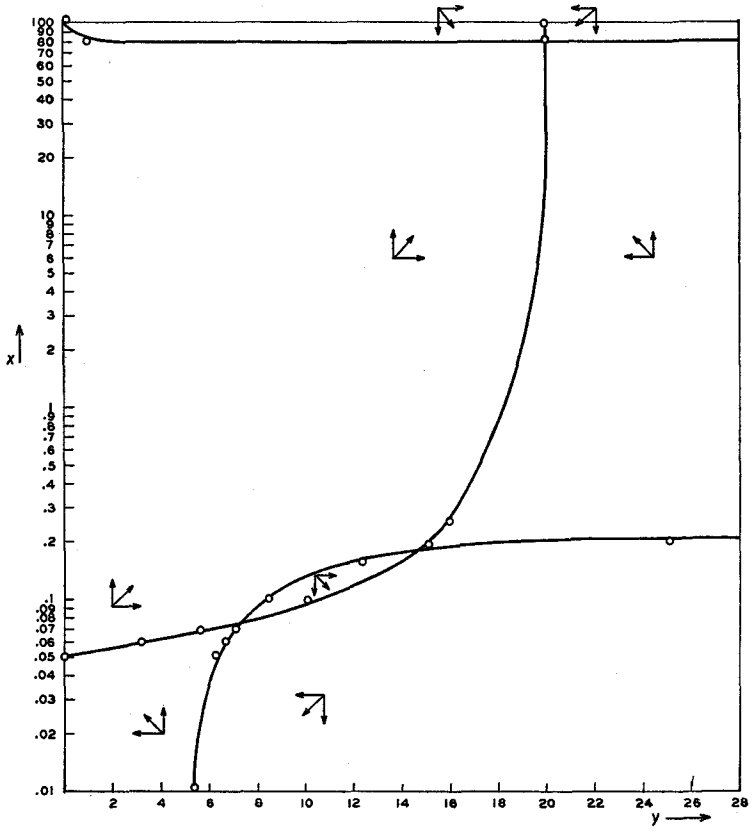

FIGURE 6. The case: $k_1 > k_3 + k_2(\sigma_1)/(\sigma_2)$. Evaluated for the values $\sigma_1 = \sigma_2 = 1$, $k_1 = 101$, $k_2 = 20$, $k_3 = k_4 = 1$.

steady state, namely, at the origin. This, therefore, represents a transient system.

2. Case: $k_3 < k_1 < k_3 + k_2(\sigma_1)/(\sigma_2)$. Here, as shown in Figure 5 and described in Appendix VA, exists one unstable root at the origin and one stable root for positive values of x and y.

3. Case: $k_1 > k_3 + k_2(\sigma_1)/(\sigma_2)$. As can be seen from Figure 6, this system has four steady states, two stable and two unstable. Hence, given this

set of parameters, multiple steady states arise for this system. The system will occupy a particular steady state according to the particular values it has on displacement.

We can use such a mechanism to suggest an explanation of the gene position effect. Let x and y be the concentrations of two enzymes produced at a certain chromosomal locus F and let x have as a precursor a metabolite produced by a gene G at another locus. Hence the parameter k_1 in the action scheme above can be equated to $w_1\mathbf{G}$, where $\mathbf{G}$ is the precursor concentration at F. This value is given by the following equation:

$$\frac{d\mathbf{G}}{dt} = \lambda(\mathbf{G}_0 - \mathbf{G}) - \frac{w_1\mathbf{G}x}{1+\sigma_1 x}, \tag{53}$$

where $\mathbf{G}_0$ is its concentration at the locus of G. In the steady state

$$= \frac{\lambda\mathbf{G}_0}{\lambda + \dfrac{w_1 x}{1+\sigma_1 x}}.$$

Also, λ is given by Q/l where Q is a constant and l the distance between G and F. Hence if the distance l is increased because of a mutation involving a change in gene positions, $\mathbf{G}$ is correspondingly decreased. But, as pointed out, k_1 is proportional to $\mathbf{G}$. The enzyme system may therefore start out in state 3 with its two possible stable steady states and, as a result of a shift in the respective distances between the two genes, the value of k_1 may be sufficiently decreased to make only state 1 or 2 possible. The system must then leave its previous steady state and drop to a lower one, including, possibly, to the zero steady state.

It can be concluded therefore that multiple steady states are possible, but require somewhat unusual kinetic systems. In particular, a combination of autocatalysis and surface reactions gives rise to two stable steady states if certain inequalities are satisfied. Such a model can be used to explain gene position effect. The required property of surface reaction again indicates the importance of the role of structural features in biology.

VI. pH *and structure.* The significance of pH in cellular systems is well appreciated and a great deal of experimental and theoretical work has been done bearing on various aspects of this question. In this paper we shall investigate the following two problems:

1. It is well known (Danielli, *loc. cit.*) that the amphoteric nature of proteins (enzymes) gives rise to a local pH near the surface of the protein which can differ markedly from the average value in the medium. We shall study the effect of enzyme aggregation on this local pH.

2. It is also well known that enzyme reactions are very sensitive to changes in pH. In particular, we shall examine the effect of enzyme localization in a 2-step system on the optimum pH for the reaction.

A. The local pH. Since enzymes are amphoteric in nature, they can possess a net charge. This net charge attracts ions from the medium to form a diffuse double layer as described by the Debye-Hückel theory. In particular, H^+ and OH^- ions will be attracted, thus giving rise to a local pH capable of differing considerably from the average.

The enzyme aggregation causing this electrostatic field can, of course, differ in extent. It seems reasonable to assume that the size of the aggregation will have an effect on the magnitude of this local pH.

We shall investigate two models. The first is represented by a sphere whose outer surface consists of a layer of charge-bearing enzymes. If we increase the radius of the sphere *and* keep the surface charge density constant, we are essentially describing a situation of increasing enzyme aggregation. Physically, perhaps such a sphere could correspond to a microsome.

We now assume that the active groups on the enzyme surface are reacting with the *local* H^+ and OH^- ions according to the following scheme

$$H^+ + RA^- \rightleftarrows RAH\,, \qquad OH^- + R'C^+ \rightleftarrows R'CO + H_2O\,,$$

where RA^- represents a surface anionic group, $R'C^+$ a surface cationic group. It therefore follows, remembering $(H^+)(OH^-) = \bar{K} = 10^{-14}$, that

$$\left.\begin{aligned}(RA^-) &= \frac{\alpha_1}{(H^+)}\\ (R'C^+) &= \alpha_2 (H^+)\,,\end{aligned}\right\} \tag{54}$$

where α_1 and α_2 are constants. Physically, this means that the surface charges of the enzymes are effected by the local pH of the medium at the same time that the local pH is determined by the surface charges.

Let x_0 be the hydrogen ion concentration in the medium, i.e., $r = \infty$, and let $\psi(r)$ be the electrostatic potential due to the charged enzyme surface. It follows then from the Boltzmann distribution that the hydrogen ion concentration at $r = a$, i.e., at the sphere surface, is given by

$$x = x_0 \exp\left[\frac{-e\psi(a)}{kT}\right];$$

hence

$$[OH^-] = \frac{\bar{K}}{x_0} \exp\left[\frac{e\psi(a)}{kT}\right].$$

Here e represents the charge of one electron, k the Boltzmann constant, and T the absolute temperature. Remembering now that the surface anions

and cations react only with the H^+ and OH^- ions at $r = a$, we can rewrite (54)

$$\left.\begin{aligned}(RA^-) &= \frac{a_1}{x_0}\exp\left[\frac{e\psi(a)}{kT}\right]\\ (R'C^+) &= a_2 x_0 \exp\left[-\frac{e\psi(a)}{kT}\right].\end{aligned}\right\} \tag{55}$$

Our problem now becomes that of finding $\psi(r)$. This can be determined from the Debye-Hückel theory. It is given by (Verwey and Overbeck, 1948)

$$\psi(r) = \frac{\psi_0}{r}\, e^{-Kr}, \tag{56}$$

where K is the well known Debye-Hückel constant. The coefficient ψ_0 is evaluated by using the relation

$$-\left(\frac{d\psi}{dr}\right)_a = \frac{4\pi}{\epsilon}\,\sigma, \tag{57}$$

where ϵ is the dielectric coefficient. The surface charge density σ is given by

$$\sigma = e\,[(R'C^+) - (RA^-)]. \tag{58}$$

From (55), (56), (57), and (58), the local pH can be determined quite simply. On the basis of the assumption underlying classical Debye-Hückel theory, namely, $[e\psi(r)]/(kT) \ll 1$, the resulting transcendental equation can be linearized. Our final expression for the local hydrogen ion concentration $[H^+](a)$ is given by (Appendix VIA)

$$[H^+](a) = x_0 \exp\left\{-\frac{4\pi e^2 a\,(a_2x_0^2 - a_1)}{kT\left[\epsilon\,(aK+1)\,x_0 + \frac{4\pi e^2}{kT}\,a\,(a_2x_0^2 + a_1)\right]}\right\}. \tag{59}$$

It is clear from (59) that if $x_0 > \sqrt{[(a_1)/(a_2)]}$, the local hydrogen ion concentration decreases with increasing radius a, and has the asymptotic value

$$[H^+](a) = x_0 \exp\left\{-\frac{4\pi e^2\,(a_2x_0^2 - a_1)}{kT\left[\epsilon K x_0 + \frac{4\pi e^2}{kT}\,(a_2x_0^2 + a_1)\right]}\right\}. \tag{60}$$

On the other hand, if $x_0 < \sqrt{[(a_1)/(a_2)]}$, the local hydrogen ion concentration increases with increasing radius a, and has the asymptotic value

$$[H^+](a) = x_0 \exp\left\{\frac{4\pi e^2\,(a_1 - a_2x_0^2)}{kT\left[\epsilon K x_0 + \frac{4\pi e^2}{kT}\,(a_2x_0^2 + a_1)\right]}\right\}. \tag{61}$$

Hence if the pH of the medium is great, the local pH will decrease with increasing enzyme aggregation; if the pH of the medium is small, the local pH will increase with increasing enzyme aggregation. These conclusions are summarized in Figure 7.

The second model which we shall investigate differs from the first in that the enzymes are assumed to be adsorbed on the inner surface of a spherical shell. The significant pH is therefore that on the inside of this sphere. Physically, this may correspond to a structure such as a mitochondrion.

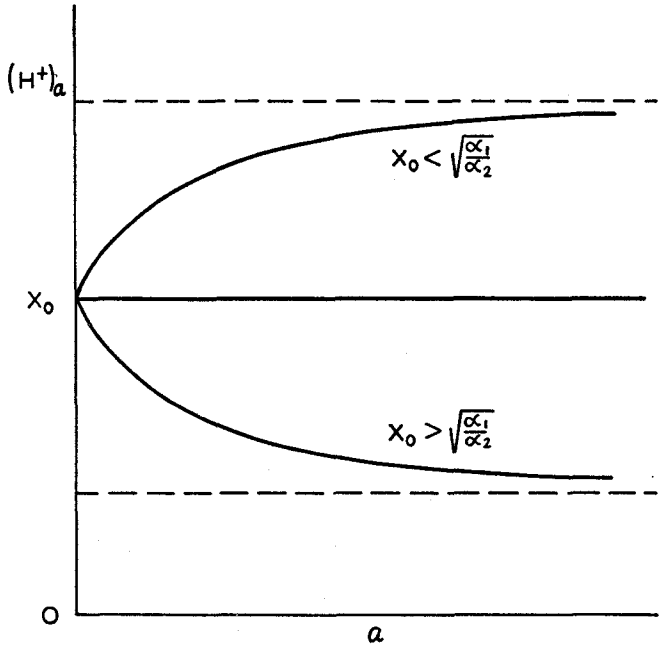

FIGURE 7. Hydrogen ion concentration vs. radius; enzyme on outer surface

The potential inside of such a sphere is given by Debye-Hückel theory (Appendix VIB) as

$$\psi(r) = \frac{\psi_0}{r} \sinh Kr, \tag{62}$$

and the constant ψ_0 is determined from

$$\left.\frac{d\psi}{dr}\right)_a = \frac{4\pi\sigma}{\epsilon}. \tag{63}$$

From (55), (62), and (63), ψ_0 can be determined. Again using the approximation $(e/kT)\ \psi(a) \ll 1$, we finally determined (Appendix VIB)

$$\psi(a) = \frac{\beta_1}{\left(\coth Ka - \frac{1}{Ka}\right) K + \beta_2}, \tag{64}$$

where

$$\left.\begin{aligned} \beta_1 &= \frac{4\pi e}{\epsilon}\left(a_2 x_0 - \frac{a_1}{x_0}\right), \\ \beta_2 &= \frac{4\pi e^2}{\epsilon kT}\left(a_2 x_0 + \frac{a_1}{x_0}\right). \end{aligned}\right\} \tag{65}$$

Since

$$\lim_{a\to 0} \psi(a) = \frac{\beta_1}{\beta_2} \quad \text{and} \quad \lim_{a\to\infty} \psi(a) = \frac{\beta_1}{K+\beta_2},$$

we can see from (64) that $\psi(a)$ is monotonically increasing for $\beta_1 > 0$ and monotonically decreasing for $\beta_1 < 0$. Since the hydrogen ion concentration is given by

$$x_0 \exp\left[-\frac{e}{kT}\psi(a)\right],$$

the following conclusions can be drawn:

1. If x_0 is less than $\sqrt{[(a_1)/(a_2)]}(\beta_1 > 0)$, the local (H^+) starts off, for $a = 0$, at

$$x_0 \exp\left[-\frac{e}{kT}\frac{\beta_1}{\beta_2}\right],$$

which is greater than X_0, and decreases to

$$x_0 \exp\left[-\frac{e}{kT}\frac{\beta_1}{K+\beta_2}\right] \quad \text{for} \quad a = \infty.$$

2. If x_0 is greater than $\sqrt{[(a_1)/(a_2)]}(\beta_1 > 0)$, the local (H^+) starts off, for $a = 0$, at

$$x_0 \exp\left[-\frac{e}{kT}\frac{\beta_1}{\beta_2}\right],$$

which is less than x_0, and increases to

$$x_0 \exp\left[-\frac{e}{kT}\frac{\beta_1}{K+\beta_2}\right].$$

These conclusions are summarized graphically in Figure 8.

The behavior of the local pH inside of a sphere is therefore considerably different from its behavior outside of a sphere.

B. pH and a 2-step enzyme system. It is well known that changes in pH greatly affect the rates of enzyme reactions. This is easily understand-

able, since a good part of enzyme-substrate interaction is probably mediated via electrostatic forces which are very much affected by the electrolyte concentration in the medium. Also, hydrogen and hydroxyl ions probably react with charged groups on the enzyme surface and hence compete with the substrate for reactive sites.

It has been shown for a good many enzyme systems that an optimum pH exists, differing of course from enzyme to enzyme, and that the reaction rate drops away quite sharply on each side of this optimum. Various

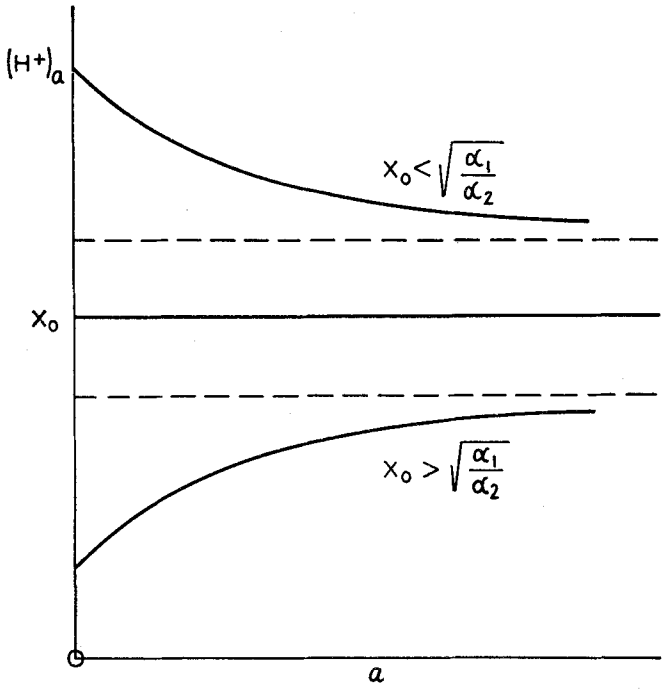

FIGURE 8. Hydrogen ion concentration vs. radius; enzyme on inner surface

theories have been advanced to account for these characteristic curves; some of them indicate that the expected optimum would be at the isoelectric point for the protein. Actually a good many of the experimental curves for enzymes place the optimum pH not at the isoelectric point but as much as 1 or 2 pH units off. This would seem to indicate that the relation between pH and enzyme action is of a more complicated nature.

We shall attempt to derive the typical bell shaped rate vs. pH curve from a simple model which does *not* require that the optimum pH be at the enzyme isoelectric point. To simplify the calculations we shall assume

here that the hydrogen and hydroxyl concentrations are uniform throughout the whole region, i.e., the Boltzmann distribution according to the electrostatic field is ignored. Our model is as follows: Assume two enzymatic surfaces placed a distance l apart, so that the first step of a reaction, $S_1 \rightarrow p_1$, occurs on the first surface. We assume that S_1 is present in large enough quantities in the medium so that its concentration can be considered constant. The substrate p_1 diffuses through the medium and reacts on the second surface to form p_2. We shall also assume that the production of p_1 occurs on negatively charged sites of surface 1, while the production of p_2 occurs on the positively charged sites of surface 2. Hence hydrogen ions will act competitively on surface 1, and hydroxyl ions will act competitively on surface 2. We shall also assume that p_1 tends to re-form S_1 on the first surface, but via a different uncharged enzyme.

The reactions taking place on the first surface are, therefore,

$$E_1 + S_1 \underset{\sigma_{-1}}{\overset{\sigma_1}{\rightleftarrows}} (ES_1) \ ,$$

$$(ES_1) \overset{\sigma_2}{\rightarrow} p_1 + E_1 \ ,$$

$$E_1 + \mathrm{H}^+ \overset{K_1}{\longleftrightarrow} (EH_1) \ ,$$

$$p_1 \overset{k_2}{\rightarrow} S_1 \ .$$

The steady-state rate of production of p_1 is then given by

$$\sigma_2 (ES_1) - k_2 p_1 (0) \ .$$

The first part of this expression, $\sigma_2(ES_1)$, follows directly from the theory of competitive inhibition (Wilson, 1949). It is given by

$$\sigma_2 (ES_1) = \frac{\sigma_1 \sigma_2 S_1 E_{1T}}{(1 + K_1 \mathrm{H}^+)\ (\sigma_2 + \sigma_{-1}) + \sigma_1 S_1} , \tag{66}$$

where E_{1T} is the total concentration of enzyme available on the first surface. If $p_1(l)$ represents the concentration of p_1 at the second surface, we have a similar reaction there, giving rise to p_2 with OH^- ions competing for the positively charged active sites. The rate of p_2 production is therefore given by

$$v_1 p_1 (l) = \frac{\sigma_3 \sigma_4 E_{2T} p_1 (l)}{(1 + K_2 \mathrm{OH}^-)\ (\sigma_4 + \sigma_{-3}) + \sigma_3 S_2} . \tag{67}$$

To simplify calculations we shall assume that $p_1(l)$ is much smaller than

$$\frac{1}{\sigma_3} (1 + K_2 \mathrm{OH}^-)\ (\sigma_4 + \sigma_{-3}) \ .$$

Hence (67) can be approximated by

$$v_2 p_1(l) = \frac{\sigma_3 \sigma_4 E_{2T} p_1(l)}{(1 + K_2 \mathrm{OH}^-)(\sigma_4 + \sigma_{-3})}. \tag{68}$$

The desired rate is given by (68). But to evaluate it we must find $p_1(l)$. This requires solving

$$\frac{d^2 p_1}{dx^2} = 0,$$

with the boundary conditions

$$-\frac{dp_1}{dx}\Big)_0 = \sigma_2 (ES_1) - k_2 p_1(0), \qquad -\frac{dp_1}{dx}\Big)_l = v_1 p_1(l).$$

These equations in p_1 are identical with (27) and (28) in B, except that

$$k_1 A = \sigma_2 (ES_1), \qquad k_3 = v_1.$$

If we again set $\mathrm{H}^+ = x$, $\mathrm{OH}^- = \sigma/x$, the steady-state rate, $v_1 p_1(l)$, using (29), can finally be written

$$v_2 = \frac{\gamma_1 x}{\gamma_2 + \gamma_3 x + \gamma_4 x^2}, \tag{69}$$

where

$$\left.\begin{aligned}
\gamma_1 &= \sigma_1 \sigma_2 \sigma_3 \sigma_4 S_1 E_{1T} E_{2T} D, \\
\gamma_2 &= D k_2 K_2 \sigma_4 (\sigma_4 + \sigma_{-3})(\sigma_2 + \sigma_{-1} + \sigma_1 S_1), \\
\gamma_3 &= (\sigma_2 + \sigma_{-1} + \sigma_1 S_1)[D k_2 (\sigma_4 + \sigma_{-3}) + \sigma_3 \sigma_4 E_{2T} (D + l k_2)] \\
&\quad + K_1 (\sigma_2 + \sigma_{-1}) \sigma_4 D k_2 K_2 (\sigma_4 + \sigma_{-3}), \\
\gamma_4 &= K_1 (\sigma_2 + \sigma_{-1})[D k_2 (\sigma_4 + \sigma_{-3}) + (D + l k_2) \sigma_3 \sigma_4 E_{2T}].
\end{aligned}\right\} \tag{70}$$

As can be seen from (69), v_3 is zero for $x = 0$ and is again zero for $x = \infty$. In particular, the optimum hydrogen ion concentration is given by

$$x_{\max} = \sqrt{\left(\frac{\gamma_2}{\gamma_4}\right)}. \tag{71}$$

It is clear from (70) that this optimum is determined not only by the parameters involving the enzyme itself, but also by the separation between the two enzymes, and the diffusion coefficient D of the substrate p_1. Hence the optimum is not necessarily at the isoelectric point but is determined, in a complicated manner, by the total system.

VII. Osmotic pressure. An integral part of studies on electrolytic solution has been the investigation of osmotic pressure due to such phenomena. One of the best known approaches to its determination has been via the Donnan equilibrium.

We shall examine this problem from our particular standpoint of enzyme aggregation. Consider a sphere of radius a, whose inner surface is covered with protein material bearing a net charge. As a result of the potential arising from the surface charge, electrolytes from the solution will be attracted to or repulsed from the region of protein concentration depending upon their sign and the sign of the net charge. If the result of this attraction and repulsion is an increase in the total number of molecules in the inner spherical region, as compared to the medium, osmotic pressure will develop and water will tend to flow into the sphere. If the total number of particles in the enzyme region is smaller, water will tend to flow out into the medium. As a first approximation, we shall use the van't Hoff equation for osmotic pressure:

$$P = kt \sum_{i=1}^{n} (\bar{n}_i - n_{io}) ,$$

where $\bar{n}_i$ represents the average number of molecules of the ith electrolyte per unit volume of sphere, and n_{i0} its number per unit volume in the medium.

Further, $n_i(r)$, $0 \leq r \leq a$, is given by $n_{10} \exp [-ez_i\psi(r)/kt]$, where z_i is the valence of the ith electrolyte. Hence the average number per unit volume is given by

$$\bar{n}_i = \frac{3}{a^3} \int_0^a r^2 n_{i0} e^{-[ez_i\psi(r)]/(kT)} dr . \tag{72}$$

This intergral can be evaluated by expanding the exponential and discarding all but the first three terms. Remembering (62), the final expression becomes (Appendix VII)

$$P = \frac{3}{4} \frac{e^2\psi_0^2}{kTa^3\lambda} \sqrt{(\Sigma z_i^2 n_{i0})} \, (\sinh 2Ka - 2Ka) , \tag{73}$$

where $K = \lambda\sqrt{(\Sigma z_i^2 n_{i0})}$. We observe from (73) that if ψ_0 is independent of the radius P becomes infinitely large as a becomes infinite.

We now modify the picture, by assuming further that the electrolytes in the medium not only form a double layer, but also react chemically with the charged groups on the enzyme surface; hence affecting the charge density and, therefore, ψ_0 also. Using (63) we can, therefore, evaluate ψ_0. As shown in Appendix VII **it** becomes

$$\psi_0 = \frac{\xi_1 a^2}{aK \cosh Ka + \sinh Ka \, (a\xi_2 - 1)} , \tag{74}$$

where

$$\xi_1 = \frac{4\pi}{\epsilon}\left[\frac{B_T^+}{\Sigma\Omega_i n_{-i}} - \frac{A_T^-}{\Sigma K_i n_{+i}}\right] \qquad \xi_2 = \frac{4\pi e}{\epsilon kT}\left[\frac{B_T^+}{\Sigma\Omega_i n_{-i}} + \frac{A_T^-}{\Sigma K_i n_{+i}}\right].$$

We can now express P as a function of a. It is given as

$$P = \frac{3}{4}\frac{e^2\xi_1^2}{kT\lambda}\sqrt{(\Sigma_i^2 n_{io})}\,\frac{a(\sinh 2Ka - 2Ka)}{[aK\cosh Ka + \sinh Ka(a\xi_2 - 1)]^2}. \qquad (75)$$

A few observations can be made about (75): When a equals zero, P is zero. When a becomes very large, P becomes zero also; hence P must have a maximum for some radius a.

It is difficult to assess how significant the actual magnitude of this osmotic pressure could become. It is of interest to read in the experimental literature (Shelton, 1953) that mitochondria, as observed in cultured fibroblasts, "assume a bewildering variety of shapes. . . . Large 'hollow' blebs appearing at the ends or in the middle of a mitochondrion would swell and collapse, frequently pinching off a piece of mitochondrion at the point of rupture."

It is tempting to speculate that perhaps these morphological changes could be due to variations in osmotic pressure, which variation might perhaps be traced to changes in charge density of mitochondrial enzymes as a result of a progressing reaction or of alterations in the size of the particles.

It also seems possible to ascribe the lysis of viral hosts to osmotic pressure resulting from the production, by the virus, of a large number of highly charged DNA molecules inside the cell. The lytic action of phage would, therefore, be due to the act of localizing electrolytes inside the host cell, hence giving rise to an increasing charge density in that volume.

VIII. Morphology. As previously mentioned, a number of investigators have found some correlation between shape of mitochondria and variations in metabolism. For this reason it is of interest to see whether any simple conclusions follow from our kinetic analysis concerning relation between shape of these cell particles and steady-state rates.

Unfortunately the limitation of the kinetic analysis to 2-step systems makes any general deduction rather dubious. Also, there are many other factors in addition to simple steady-state rates which play significant roles in this relation and which are not considered here.

Considering rate only, then, we see that the rate in a certain region will be very much determined by the amount of diffusion possible, and this, in turn, will depend on the total surface area available for outward diffusion. The sphere having the smallest surface for a given volume will have mini-

mum diffusion. In the case of a 1-step open system with one diffusible intermediate, this becomes the only significant effect and therefore the rate of the sphere will be minimum, as compared, for example, to the rate in a cylinder of equal volume.

Consider now a 2-step system with two diffusible intermediates of the form

$$\underset{K_{-1}}{\overset{K_1}{\rightleftarrows}} C \underset{K_{-2}}{\overset{K_2}{\rightleftarrows}} C_2 .$$

Here the situation is somewhat different. If the surface area is too great, all of the first intermediate C_1 diffuses out of the system and the second step never occurs. Hence there exists an optimum surface area for a given volume, as distinct from the previous case where the optimum was the maximum surface area possible.

This can be seen more concretely by using Landahl's (1953) approximation method. The rate of appearance of C_2 in a sphere is given by (Appendix VIII)

$$W_1 = \frac{16 K_1 K_2 \pi D R^5}{144 D^2 + 12 D R^2 (K_{-1} + K_2 + K_{-2}) + K_{-1} K_{-2} R^4}, \qquad (76)$$

where D is the diffusion coefficient and R the radius of the sphere. In the case of a cylinder of volume $V = \frac{4}{3}\pi R^3 = \pi h_0^2 z_0$, the rate of appearance of C_2 is given by (Appendix VIII)

$$W_2 = \frac{6\pi D V^6 n_o^2 (4h_o^6 + V^2)}{P}, \qquad (77)$$

where

$$P = [124 D h_o^6 + 6 D V^2 + K_{-2} V^2 h_o^2]\ [24 D h_o^6 + 6 D V^2 + K_{-1} + K_2 V^2 h_o^2] - K_{-2} K_2 h_o^4 V^4 .$$

Clearly W_2 is zero for both $h_0 = 0$ and very large h_0. Hence an optimum value of h_0 exists, as expected. Further, there will exist at least two ranges of h_0 for which the rate of the sphere is larger than that in the cylinder. It is also possible that the rate in the sphere is greater than that in the cylinder for any value of h_0. It is clear, therefore, that the conclusions drawn from a 1-step system are not applicable to higher order systems.

IX. Discussion and conclusions. It has been the purpose of this paper to examine the significance of structure of enzyme systems. The biological importance of this problem can hardly be overestimated. It is becoming more and more apparent as a result of biological research that life means organization and pattern—not only chemically, not only in terms of a

given set of reactions, but also in terms of spatial and geometric relation, linking together chemical reactions, bioelectric potentials, mechanical forces, and all the many processes characteristic of living things.

It is becoming apparent that these structures must be studied not only statically, although this is of undeniable importance, but dynamically, in their relation to the ongoing activities of the cell. This sentiment is well known today and needs little discussion. But it is not too clear how this problem should best be attacked, and most of our knowledge concerning the relation of organization to function is still on a far too vague level.

This paper was conceived as an attempt at a particular approach toward this basic problem. In all cases attention was focused on how to relate a specific biological, chemical, and physical process or property to a given structure, or changes in structure. The purpose is frankly exploratory; the author does not believe that biology is at the stage where penetrating theoretical developments can occur apart from an integrated operation involving experimental *and* theoretical work.

On the other hand, such theoretical work can open up lines of thought and indicate relations which otherwise might be missed. It also offers opportunity for collecting diverse data and arranging them in a semblance of order. Further, it develops models and their consequences which can be of significance to the field.

This paper was devoted to a series of questions, all focusing on the problem of the relation of structure to activity. The first question concerned itself with the effect of enzyme compartmentalization on the steady state of rate of reactions. It appears that for the particular kinetic system studied, the delaying effect of diffusion is dominant, and therefore that the steady-state rate is decreased as a result of this kind of compartmentalization. This result led quite naturally to an examination of the validity of the kinetic scheme used. It then appeared that the conclusion was perhaps of dubious validity because the basic model was biologically inadequate. It was suggested that perhaps the more fitting model for reactions inside of cells, in particular inside of particles, would be surfaces immersed in solution. A very simple examination of this model indicated that here compartmentalization does lead to an increase in the steady-state rate.

Another significant problem dealt with was that of access of substrate to enzyme. It was shown that a simple localization of enzymes with respect to the enzyme source can give rise to important effects on the competitive situation, and assist the channelization of reactions one way or the other. The effect of enzyme localization was also studied with respect to the steady-state concentration of intermediate, and it was shown that in gen-

eral the non-uniform concentration will be larger than the uniform, hence also assisting competition.

We also dealt with the question of possible periodicities. It was established that the simple kind of kinetic systems, both uniform and non-uniform, do not satisfy the sufficient criterion for periodicity. Further, in the case of autocatalytic reactions, diffusion which enters as a factor in the compartmentalized system seems to reduce the probability of periodicity.

The phenomenon of overshoot was also examined and it was shown that structural features, if involved in reaction systems, provide additional parameters which can lead to overshoot. Furthermore, it was shown that the conditions for overshoot in terms of variations in the system parameters are different for uniform and non-uniform systems.

Some space was devoted to multiple steady states, a subject of biological interest which has been insufficiently investigated. It was shown that a number of systems which commonly occur do not possess this property, but that a combined surface and autocatalytic system does. A possible mechanism whereby such multiple steady states could give rise to gene position effects was postulated.

The problem of pH was then investigated, again with particular focus on the relation between pH and structure. It was shown that the local pH in the neighborhood of amphoteric enzyme systems is affected by the extent of enzyme aggregation. The nature of this effect depends on whether the enzymes are spread on the inside or outside surface of the particle. Specific relations between changes in aggregation and local pH were established.

Another well known problem which was investigated with reference to structure was the relation of reaction rate to pH and the nature of the optimum pH. In particular, a 2-step surface system was studied in which the reactions were assumed to occur via differently charged groups. It was shown that the reaction rate, when plotted against the pH, showed the characteristic curve, and that the optimum pH was not at the isoelectric point, but determined by the parameters of the whole system.

The problem of osmotic pressure effects resulting from charged surfaces was also examined. The expression for the osmotic pressure inside of a sphere of uniform charge density was deduced; it was shown that the osmotic pressure has an optimum radius, i.e., it has a maximum for a particular value and decreases on both sides of this optimum. Some possible relations to biological phenomena were postulated.

Finally, a short examination was made of the relation of the shape of the particle (reaction volume) to the reaction rate. It was shown that for the

1-step system the sphere has the slowest rate, but that in 2-step systems the sphere can have larger rates than the cylinder.

On the basis of these considerations it may be concluded that structure as studied has significant effects on important biological properties.

I wish to thank the Committee on Mathematical Biology and, in particular, Professor H. D. Landahl and Dr. C. S. Patlak for their help and criticism.

(Note: Appendices IA, IB, IVA, IVB, etc. following are elaborations of Sections I, IV, etc. in text.)

APPENDIX IA

If $\lambda = 0$ in (7), v_1 becomes q_1. We want to determine the ratio q_1/v_1. Since the problem of finding ratios will occur frequently, we shall simplify the operation as follows. Let

$$W_1 = \frac{A_1 + B_1}{C_1 + D_1} \qquad W_2 = \frac{A_1 + B_2}{C_1 + D_2}. \tag{78}$$

Hence, taking the ratio

$$\frac{W_1}{W_2} = \frac{(A_1 + B_2 - B_2 + B_1)\ (C_1 + D_1 - D_1 + D_2)}{(C_1 + D_1)\ (A_1 + B_2)} \tag{79}$$

or

$$\frac{W_1}{W_2} = 1 + \frac{B_1 (C_1 + D_2) + A_1 (D_2 - D_1) - B_2 (D_1 + C_1)}{(C_1 + D_1)\ (A_1 + B_2)}. \tag{80}$$

We shall use formula (80) in all future calculations of ratios.

In this particular case, setting $q_1 = W_1$, $v_1 = W_2$, we see that

$$\left.\begin{aligned} &B_1 = 0 = D_1\,, && A_1 = w_1 X_0 w_2 - w_{-1} w_{-2} X_2\,, \\ &B_2 = \lambda\,(w_1 X_0 - w_{-1} X_{-10})\,, && C_1 = w_2 + w_{-1}\,, \qquad D_2 = \lambda\,. \end{aligned}\right\} \tag{81}$$

Hence substitution of (81) into (80) results in

$$\frac{q}{v_1} = 1 + \frac{w_{-1}\lambda\,[X_{10}\,(w_2 + w_{-1}) - w_1 X_0 - w_{-2} X_2]}{(w_2 + w_{-1})\ [w_1 X_0\,(w_2 + \lambda) - w_{-1}\lambda X_{10} - w_{-1} w_{-2} X_2]}. \tag{82}$$

Equation (82) is 1 when

$$X_{10} = \frac{w_1 X_0 + w_{-2} X_2}{w_2 + w_{-1}};$$

it is infinite when

$$X_{10} = \frac{w_1 X_0\,(\lambda + w_2) - w_{-1} w_{-2} X_2}{w_{-1}\lambda}.$$

Since the latter is larger than the former, the conclusions in the text follow.

APPENDIX IB

The basic equation to be solved is

$$\frac{d^2B}{dx^2} = 0 , \tag{83}$$

with boundary conditions

$$\left.\begin{aligned} -D\frac{dB}{dx}\Big)_0 &= k_1A - k_2B(0) , \\ -D\frac{dB}{dx}\Big) &= k_3B(l) . \end{aligned}\right\} \tag{84}$$

The solution of (83) is given by

$$B(x) = c_1 + c_2x . \tag{85}$$

The coefficients c_1 and c_2 are determined from (84). They are

$$\left.\begin{aligned} c_2 &= \frac{-k_1k_3A_1}{D(k_2+k_3)+k_2k_3l} , \\ c_1 &= \frac{k_1A_1(D+k_3l)}{D(k_2+k_3)+k_2k_3l} . \end{aligned}\right\} \tag{86}$$

Hence

$$B(l) = \frac{Dk_1A_1}{D(k_2+k_3)+k_2k_3l} ; \tag{87}$$

(28) follows from (87) directly.

APPENDIX IVA

We have

$$\left.\begin{aligned} &E_1 + S_1 \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} X_1 , \\ &X_1 \underset{\sigma_{-1}}{\overset{\sigma_1}{\rightleftarrows}} S_2 + E_1 \\ &\cdot \\ &\cdot \\ &\cdot \\ &X_n \underset{\sigma_{-n}}{\overset{\sigma_n}{\rightleftarrows}} S_{n+1} + E_n , \end{aligned}\right\} \tag{88}$$

with these two conditions

$$\left.\begin{aligned} E_{oi} &= X_i + E_i , \qquad i = 1, 2, \ldots n \\ K &= \sum_{i=1}^{n} (S_i + X_i) + S_{n+1} . \end{aligned}\right\} \tag{89}$$

Hence

$$S_1 = K - X_1 - \sum_{2}^{n} (S_i + X_i) - S_{n+1} .$$

The basic equations describing (88) are

$$\left.\begin{aligned}
\frac{dX_1}{dt} &= k_1E_1S_1 - X_1(k_{-1}+\sigma_1) + \sigma_{-1}S_2E_1 \\
\frac{dS_2}{dt} &= \sigma_1X_1 - \sigma_{-1}S_2(E_{01}-X_1) - k_2S_2(E_{02}-X_2) + k_{-2}X_2 \\
&\cdot \\
&\cdot \\
&\cdot \\
\frac{dS_{n+1}}{dt} &= \sigma_nX_n - \sigma_{-n}S_{n+1}E_{0n} + \sigma_{-n}S_{n+1}X_n .
\end{aligned}\right\} \quad (90)$$

Setting

$$\alpha_1 = X_i - \bar{X}_i , \qquad i = 1, 2, \ldots n ,$$

$$\beta_1 = S_i - \bar{S}_i , \qquad i = 2, 3, \ldots n+1 ,$$

eliminating all terms in α_i or β_i higher than first power, and using (89), results in

$$\left.\begin{aligned}
\frac{d\alpha_1}{dt} &= \alpha_1\Big[k_1\sum_1^n \bar{X}_i + k_1\bar{X}_1 - k_1K + k_1\sum_2^{n+1}\bar{S}_i - k_1E_{10} \\
&\quad - \sigma_1 - k_{-1} - \sigma_{-1}\bar{S}_2\Big] + \beta_2\left[k_1\bar{X}_1 - k_1E_{10} + \sigma_{-1}E_{10} - \sigma_{-1}\bar{X}_1\right] \\
&\quad - k_1\sum_3^{n+1}\beta_i(E_{10}-\bar{X}_1) - k_1\sum_2^n\alpha_i(E_{10}-\bar{X}_1) , \\
\frac{d\beta_2}{dt} &= \alpha_1(\sigma_1+\sigma_{-1}\bar{S}_2) + \beta_2(\sigma_{-1}\bar{X}_1 - \sigma_{-1}E_{01} - k_2E_{02} + k_2\bar{X}_2) \\
&\quad + \alpha_2(k_2\bar{S}_2 + k_{-2}) \\
&\cdot \\
&\cdot \\
&\cdot \\
&\cdot \\
\frac{d\alpha_j}{dt} &= \beta_jk_j(E_{oj}-\bar{X}_j) - \alpha_j(\sigma_{-j}\bar{S}_{j+1} + k_{-j} + \sigma_j + k_j\bar{S}_j) \\
&\quad + \beta_{j+1}\sigma_{-j}(E_{0j}-\bar{X}_j) , \\
\frac{d\beta_j}{dt} &= \alpha_{j-1}(\sigma_{j-1} + \sigma_{-(j-1)}\bar{S}_j) + \beta_j(\sigma_{-(j-1)}\bar{X}_{j-1} - \sigma_{-(j-1)}E_{0j-1} \\
&\quad - k_jE_{0j} + k_j\bar{X}_j) + \alpha_j(k_j\bar{S}_j + k_{-j}) .
\end{aligned}\right\} \quad (91)$$

It can be seen on inspection of (91) that there is only one pair, α_{ij}, α_{ji}, which may have opposite signs. This occurs for the coefficient of β_2 in the

first equation and for the coefficient of a_1 in the second equation. They are

$$(E_{10} - \bar{X}_1)\ (\sigma_{-1} - k_1) \qquad \text{and} \qquad \sigma_1 + \sigma_{-1}\bar{S}_2\ ,$$

respectively. Clearly if $k_1 > \sigma_{-1}$, they are of opposite sign.

APPENDIX IVB

From (90), if $n = 1$,

$$\left.\begin{aligned} K = S_2 + S_1 + X_1\ , \qquad E_1 = E_{10} - X_1\ , \\ \frac{dX_1}{dt} = k_1 (E_{01} - X_1)\ (K - S_2 - X_1) \\ - X_1 (k_{-1} + \sigma_1) + \sigma_{-1} S_2 (E_{01} - X_1)\ , \\ \frac{dS_2}{dt} = \sigma_1 X_1 - \sigma_{-1} S_2 (E_{01} - X_1)\ . \end{aligned}\right\} \quad (92)$$

We set

$$a_1 = X_1 - \bar{X}_1\ ,$$

$$\beta_2 = S_2 - \bar{S}_2\ .$$

Hence

$$\frac{da_1}{dt} = k_1 (E_{01} - a_1 - \bar{X}_1)\ (K - \beta_2 - \bar{S}_2 - a_1 - \bar{X}_1) - (a_1 + \bar{X}_1)\ (k_{-1} + \sigma_1) + \sigma_{-1} (\beta_2 + \bar{S}_2)\ (E_{01} - a_1 - \bar{X}_1)\ ,$$

$$\frac{d\beta_2}{dt} = \sigma_1 (a_1 + \bar{X}_1) - \sigma_{-1} (\beta_2 + \bar{S}_2)\ (E_{01} - a_1 - \bar{X}_1)\ .$$

Since

$$k_1 (E_{01} - \bar{X}_1)\ (K - \bar{S}_2 - \bar{X}_1) - \bar{X}_1 (k_{-1} + \sigma_1) + \sigma_{-1}\bar{S}_2 (E_{01} - \bar{X}_1) = 0\ ,$$

$$\sigma_1 \bar{X}_1 - \sigma_{-1}\bar{S}_2 (E_{01} - \bar{X}_1) = 0\ ,$$

we find

$$\frac{da_1}{dt} = -k_1 E_{01} (\beta_2 + a_1) + k_1 \bar{X}_1 (a_1 + \beta_2) - k_1 a_1 (K - \bar{S}_2 - \bar{X}_1) - a_1 (k_{-1} + \sigma_1) + \sigma_{-1}\beta_2 (E_{01} - \bar{X}_1) - \sigma_{-1}\bar{S}_2 a_1\ ,$$

$$\frac{d\beta_2}{dt} = a_1\sigma_1 - \sigma_{-1}\beta_2 (E_{01} - \bar{X}_1) + \sigma_{-1}\bar{S}_2 a_1\ .$$

We now let $a_1 = \mu_1 e^{-\lambda t}$ and $a_2 = \mu_2 e^{\lambda t}$; the above becomes

$$\left.\begin{aligned} \mu_1 [-\lambda + k_1 E_{01} - k_1 \bar{X}_1 + k_1 (K - \bar{S}_2 - \bar{X}_1) + k_{-1} + \sigma_1 + \sigma_{-1}\bar{S}_2] \\ + \mu_2 (E_{01} - \bar{X}_1)\ (k_1 - \sigma_{-1})\ = 0\ , \\ -\mu [\sigma_1 + \sigma_{-1}\bar{S}_2] + \mu_2 [-\lambda + \sigma_{-1} (E_{01} - \bar{X}_1)]\ = 0\ . \end{aligned}\right\} \quad (93)$$

Equation (44) results from setting the determinant of coefficients of (93) equal to zero and expanding.

We now calculate $b^2 - 4ac = G$, as defined in the test. It is given by

$$G = [\sigma_{-1}(\bar{E}_1 + \bar{S}_2) + k_1(\bar{E}_1 + \bar{S}_1) + \sigma_1 + k_{-1}]^2 - 4\bar{E}_1[\sigma_{-1}k_{-1} + k_1\sigma_1 + \sigma_{-1}k_1(\bar{E}_1 + \bar{S}_1 + \bar{S}_2)], \tag{94}$$

where we use the relations

$$K = \bar{S}_1 + \bar{S}_2 + \bar{X}_1,$$
$$\bar{E}_{01} = \bar{E}_1 + \bar{X}_1.$$

Equation (94) can now be expanded and simplified. The resulting equation is now written by grouping all terms in the powers of $\bar{E}_1$. The following results:

$$G = \bar{E}_1^2(\sigma_{-1} - k_1)^2 + 2\bar{E}_1(\sigma_{-1} - k_1)[\sigma_{-1}\bar{S}_2 - k_1\bar{S}_1 + \sigma_1 - k_{-1}] + (\sigma_{-1}\bar{S}_2 + k_1\bar{S}_1)^2 + (\sigma_1 + k_{-1})^2 + 2(\sigma_1 + k_{-1})(\sigma_{-1}\bar{S}_2 + k_1\bar{S}_1).$$

Inspection discloses that G is greater or equal to F, where F is given by

$$F = [\bar{E}_1(\sigma_1 - k_1) + \sigma_{-1}\bar{S}_2 - k_1\bar{S}_1 + \sigma_1 - k_{-1}]^2.$$

Since $F \geq 0$, G can never be negative.

APPENDIX IVC

This system is described by the following set of differential equations

$$\left.\begin{aligned}
&\frac{dA_{11}}{dt} = kA_{11} - r_1(A_{11} - A_{12}) - \sigma_{11}A_{11},\\
&\frac{dA_{12}}{dt} = r_1(A_{11} - A_{12}) - k_1A_{12}A_{22} - \sigma_{12}A_{12}\\
&\cdot\\
&\cdot\\
&\cdot\\
&\cdot\\
&\frac{dA_{j,j+1}}{dt} = r_j(A_{jj} - A_{j,j+1}) - k_jA_{j,j+1}A_{j+1,j+1} - \sigma_{j,j+1}A_{j,j+1},\\
&\frac{dA_{j+1,j+1}}{dt} = k_jA_{j,j+1}A_{j+1,j+1} - r_{j+1}(A_{j+1,j+1} - A_{j+1,j+2}) - \sigma_{j+1,j+1}A_{j+1,j+1}\\
&\cdot\\
&\cdot\\
&\cdot\\
&\frac{dA_{n,n}}{dt} = k_{n-1}A_{n-1,n}A_{nn} - r_nA_{nn}.
\end{aligned}\right\} \tag{95}$$

Following the usual procedure, we set $a_{ij} = A_{ij} - \bar{A}_{ij}$, where A_{ij} is the steady-state value. Hence, ignoring all but terms a_{ij} to the first power, (95) becomes

$$\left.\begin{aligned}
\frac{da_{11}}{dt} &= a_{11}(K - r_1 - \sigma_{11}) + r_1 a_{12}, \\
\frac{da_{12}}{dt} &= r_1 a_{11} - a_{12}(r_1 + k_1\bar{A}_{22} + \sigma_{12}) - k_1\bar{A}_{12}a_{22}. \\
\frac{da_{j,j+1}}{dt} &= r_j a_{jj} - a_{j,j+1}(r_j + k_j\bar{A}_{j+1,j+1} + \sigma_{j,j+1}) \\
&\qquad - a_{j+1,j+1}k_j\bar{A}_{j,j+1}, \\
\frac{da_{j+1,j+1}}{dt} &= a_{j,j+1}k_j\bar{A}_{j+1,j+1} - a_{j+1,j+1}(r_{j+1} - \sigma_{j+1,j+1} \\
&\qquad - k_j\bar{A}_{j,j+1}) + r_{j+1}a_{j+1,j+2} \\
&\cdots \\
\frac{da_{n,n}}{dt} &= a_{n-1,n}k_{n-1}\bar{A}_{n,n} - a_{n,n}(r_n - k_{n-1}\bar{A}_{n-1,n}).
\end{aligned}\right\} \tag{96}$$

Further, since $K\bar{A}_{11} - r_1(\bar{A}_{11} - \bar{A}_{12}) - \sigma_{11}\bar{A}_{11} = 0$ in the first line in (95),

$$K - r_1 - \sigma_{11} = \frac{-r_1\bar{A}_{12}}{\bar{A}_{11}} = Q_1.$$

Continuing for the second line in (95) gives us

$$r_1 + k_1\bar{A}_{22} + \sigma_{12} = \frac{r_1\bar{A}_{11}}{\bar{A}_{12}} = Q_2.$$

In general, there are $2_{n-1}Q_i$ such that

$$\left.\begin{aligned}
Q_{2j} &= \frac{r_j\bar{A}_{jj}}{\bar{A}_{j,j+1}}, \\
Q_{2j-1} &= \frac{r_j\bar{A}_{j,j+1}}{\bar{A}_{jj}}.
\end{aligned}\right\} \tag{97}$$

Hence using (96) in (97) we find that the coefficient of λ^{2n-2} is given by

$$a_1 = \sum_{1}^{2n-1} Q_j,$$

and the coefficient of λ^{2n-3} is given by

$$a_2 = \frac{1}{2}\sum_{i,j=1}^{2n-1} Q_i Q_j - \sum_{1}^{2n-1} Q_i^2 - \sum_{1}^{n-1} r_j^2 + \sum_{1}^{n-1} k_j^2 \bar{A}_{j,j+1}\bar{A}_{j+1,j+1}.$$

Therefore

$$Q_1^2 - 2a_2 = \sum_{1}^{2n-1} Q_i^2 + 2\sum_{1}^{n-1} r_j^2 - 2\sum_{1}^{n-1} k_j^2 \bar{A}_{j,j+1}\bar{A}_{j+1,j+1}. \tag{98}$$

Equation (98) must be less than or equal to zero to satisfy the sufficient condition for periodicity.

In particular, we can substitute (97) into (98). This gives

$$a_1^2 - 2a_2 = \sum_{1}^{n-1}\left(r_j^2 \frac{\bar{A}_{jj}^2}{\bar{A}_{j,j+1}^2} + r_j^2 \frac{\bar{A}_{j,j+1}^2}{\bar{A}_{jj}^2}\right) + 2\sum_{1}^{n-1} r_j^2 - 2\sum_{1}^{n-1} k_j^2 \bar{A}_{j,j+1}\bar{A}_{j+1,j+1},$$

$$= \sum_{1}^{n-1}\left\{r_j^2\left[\frac{2\bar{A}_{j,j+1}^2\bar{A}_{jj}^2 + \bar{A}_{jj}^4 + \bar{A}_{j,j+1}^4}{(\bar{A}_{jj+1}\bar{A}_{jj})^2}\right] - 2k_j^2\bar{A}_{j,j+1}\bar{A}_{j+1,j+1}\right\}.$$

Clearly, $a_1^2 - 2a_2 \leq 0$ if each term in the series obeys this inequality. This requires the inequality of (47).

APPENDIX IVD

The differential equations describing the system become, in χ_1 and χ_2,

$$\left.\begin{aligned} \frac{d\chi_1}{dt} &= -(\lambda + w_{-1})\chi_1 + \lambda\chi_2, \\ \frac{d\chi_2}{dt} &= \lambda\chi_1 - \chi_2(\lambda + w_2). \end{aligned}\right\} \tag{99}$$

The characteristic equation of (99) is $(\lambda + w_{-1}\gamma)(\lambda + w_2 - \gamma) - \lambda^2 = 0$; hence, solving this quadratic equation in γ_1 results in (49).
Setting

$$\chi_{10} = \mu_1 + \mu_2 \qquad \chi_{20} = \beta_1 + \beta_2$$

from (48), and substituting in (99) gives us

$$\left.\begin{aligned} -\gamma_1\mu_1 &= -(\lambda + w_{-1})\mu_1 + \lambda\beta_1 \\ -\gamma_2(\chi_{10} - \mu_1) &= -(\lambda + w_{-1})(\chi_{10} - \mu_1) + \lambda(\chi_{20} - \beta_1). \end{aligned}\right\} \tag{100}$$

We can solve (100) for μ_1 and β_1. In particular, equations (50) result.

Further, $-(\mu_1)/(\mu_2)$ from (50) is equal to

$$\frac{-\mu_1}{\mu_2} = 1 - \frac{\chi_{10}(\gamma_1 - \gamma_2)}{\lambda\chi_{20} - \chi_{10}(\lambda + w_{-1} - \gamma_1)}. \tag{101}$$

Since, by assumption, $\gamma_1 > \gamma_2$, (101) is only greater than 1 if

$$\lambda + w_{-1} - \gamma_1 > \frac{\lambda\chi_{20}}{\chi_{10}}.$$

We can now use (49) in the above inequality, remembering that γ_1 is the larger of the two roots. Equation (51) follows immediately. Hence, $(\chi_{20})/(\chi_{10})$ must be less than zero.

APPENDIX VA

The basic equations of this system are

$$\left.\begin{aligned} \frac{dx}{dt} &= \frac{k_1 x}{1+\sigma_1 x} - \frac{k_2 xy}{1+\sigma_2 xy} - k_3 x, \\ \frac{dy}{dt} &= \frac{k_2 xy}{1+\sigma_2 xy} - k_4 y. \end{aligned}\right\} \tag{102}$$

We now proceed to find the curves $y_1 = y_1(x)$ and $y_2 = y_2(x)$ for which dx/dt and dy/dt are zero. It can be seen immediately that for $x = 0 = y$ this condition is satisfied. Hence the origin is one of the steady-state points of the system.

Setting each equation of (102) equal to zero and solving for y results in the following two equations:

If $dx/dt = 0$, then

$$y = \frac{k_1 - k_3 - k_3\sigma_1 x}{k_3\sigma_1\sigma_2 x^2 + k_2 + x(k_3\sigma_2 + k_2\sigma_1 - k_1\sigma_2)}. \tag{103}$$

If $dy/dt = 0$, then

$$y = \frac{k_2 x - k_4}{k_4\sigma_2 x}. \tag{104}$$

Now consider the case where $k_1 < k_3$. Therefore the denominator is positive and the numerator negative in (103). The resulting curve and its intersection with the curve (104) is shown in Figure 4. Hence there is only one steady state, the origin. This is also stable, as can be seen by examining (102).

If $k_3 < k_1 < k_3 + k_2\sigma_1/\sigma_2$, the numerator is positive for small values of x, and the denominator is still positive. The resulting graph is shown in Figure 5. As can be seen from (104), the origin is no longer stable.

If $k_1 > k_3 + k_2\sigma_1/\sigma_2$, the numerator is positive for small values of x and the denominator is now a quadratic equation in x with two changes in sign. Hence it can have two roots, i.e., it can be zero for two values of x. The resulting graph is given in Figure 6. As can be seen, four steady states are now possible, two of which are stable, two unstable.

APPENDIX VIA

We see from (56) that

$$\left(\frac{-d\psi}{dr}\right)_a = \frac{\psi_0 e^{-Ka}}{a^2}(aK+1) = \frac{4\pi}{\epsilon}\sigma \tag{105}$$

and, from (55) and (58),

$$\sigma = e\left\{a_2 X_0 \exp\left[\frac{-e\psi(a)}{kT}\right] - \frac{a_1}{x_0}\exp\left[\frac{e\psi(a)}{kT}\right]\right\}.$$

We now approximate each exponential by the first two terms of its Taylor expansion. Equation (105) therefore becomes

$$\frac{\psi_0 e^{-Ka}}{a} = \frac{\frac{4\pi e}{\epsilon}\left(a_2 x_0 - \frac{a_1}{x_0}\right)}{\frac{(aK+1)\,x_0}{a} + \frac{4\pi e^2}{\epsilon kT}\left(a_2 x_0 + \frac{a_1}{x_0}\right)}. \tag{106}$$

Since

$$(H^+) = x = x_0 \exp\left(-\frac{e}{kT}\frac{\psi_0}{a}e^{-Ka}\right),$$

(59) follows directly from (106).

APPENDIX VIB

The Debye-Hückel theory for a spherically symmetrical system is given by

$$\frac{d^2 r\psi}{dr^2} = K^2 r\psi.$$

In the case of the sphere, one boundary condition is given by the requirement that ψ remain finite at the origin. This is fulfilled only if

$$\psi = \frac{\psi_0 \sinh Kr}{r}. \tag{107}$$

We now use (107) to solve for

$$\frac{d\psi}{dr}\Big)_a$$

and set this equal to $4\pi\sigma/\epsilon$. This becomes

$$\psi_0 = \frac{4\pi}{\epsilon} \frac{a^2\sigma}{(aK \cosh Ka - \sinh Ka)} . \tag{108}$$

Proceeding as before we find σ_1 and expand the exponentials in a Taylor series, ignoring all but the first two terms. Equation (64) then follows.

APPENDIX VII

The average number of molecules of type i per unit volume of the sphere is given by (72). Now

$$e^{-[ez_i\psi(r)]/kT} = \left[1 - \frac{ez_i\psi(r)}{kT} + \frac{1}{2}\left(\frac{ez_i}{kT}\right)^2\psi^2 - + \ldots\right];$$

hence, since

$$\left.\begin{aligned} P &= kT\sum_{i=1}^{n} (\bar{n}_i - n_{i0}) , \\ P &= kT\sum_{i=1}^{n} \left\{\frac{3}{a^3}\int_0^a r^2 n_{i0} \right. \\ &\qquad \left. \times\left[1 - \frac{ez_i\psi(r)}{kT} + \frac{1}{2}\left(\frac{ez_i}{kT}\right)^2\psi^2(r)\right]dr - n_{i0}\right\} . \end{aligned}\right\} \tag{109}$$

Or

$$P = kT\sum_{i=1}^{n}\left\{\frac{a}{a^3}\left[\frac{n_{i0}a^3}{3} - n_{i0}\int_0^a r^2\left(\frac{ez_i\psi}{kT} - \left[\frac{ez_i}{kT}\right]^2\frac{\psi^2}{2}\right)dr\right] - n_{i0}\right\} . \tag{110}$$

We can see that the first and last term cancel. Also, from the condition of electroneutrality,

$$\Sigma n_{i0} z_i = 0 ;$$

hence the linear term under the integral sign vanishes. The result is

$$P = \frac{3}{2}\frac{e^2}{kTa^3}\sum_{i=1}^{n} n_{i0} z_1^2 \int_0^a \psi^2 r^2 dr . \tag{111}$$

We integrate (111), using

$$\psi = \frac{\psi_0}{r}\sinh Kr ,$$

where

$$K = \lambda\sqrt{(z_i^2 n_{i0})} , \qquad \lambda = \sqrt{\frac{4\pi e^2}{\epsilon kT}} ;$$

the result is equation (73).

Further, let A^- be the anion surface charge of the enzyme, B^+ the cation

surface charge. Let C_{+i} be the ith cation in solution C_{-i} the ith anion in solution. Hence

$$\left.\begin{aligned} &A^- + C_{+1} \overset{K_1}{\longleftrightarrow} AC_{+1} \\ &\cdot \\ &\cdot \\ &\cdot \\ &A^- + C_{+n} \overset{K_n}{\longleftrightarrow} AC_n\,, \\ &B^+ C_{-1} \overset{\Omega_1}{\longleftrightarrow} BC_{-1} \\ &\cdot \\ &\cdot \\ &\cdot \\ &B^+ + C_{-n} \overset{\Omega_n}{\longleftrightarrow} BC_{-n}\,. \end{aligned}\right\} \tag{112}$$

If A_T^- = the total anionic surface concentration and B_T^+ = the total cationic surface concentration, then

$$\left.\begin{aligned} A^- &= \frac{A_T^-}{1 + \Sigma K_i C_{+i}} \\ B^+ &= \frac{B_T^+}{1 + \Sigma \Omega_i C_{-i}}\,. \end{aligned}\right\} \tag{113}$$

Further, the total surface charge $\sigma = B^+ - A^-$. Since

$$C_{+1} = n_{+i} e^{-(e\psi)/(k\,T)}\,, \qquad C_{-i} = n_{-i} e^{(e\psi)/(k\,T)}\,,$$

the surface charge can be written approximately as

$$\sigma = \frac{B_T^+ e^{-[e\psi(a)]/k\,T}}{\Sigma \Omega_i n_{-i}} - \frac{A_T^- e^{[e\psi(a)]/k\,T}}{\Sigma K_i n_{+i}}\,. \tag{114}$$

Since

$$\frac{d\psi}{dr}\Big)_a = \frac{4\pi\sigma}{\epsilon}\,,$$

we can solve for the unknown coefficient ψ_0. Approximating $\psi(a)$ in (114) by the first two terms of the Taylor expansion results in (74).

APPENDIX VIII

We assume that

$$c_1(r) = \bar{c}_1\left(1 - \frac{r}{R}\right),$$

$$c_2(r) = \bar{c}_2\left(1 - \frac{r}{R}\right),$$

where $\bar{c}_1$ and $\bar{c}_2$ are the concentrations at the center of the sphere of radius R. We now equate the total production of each substance to its outflow. Hence

$$4\pi\int_0^R \left\{ K_1 - (K_{-1}+K_2)\,\bar{c}_1\left(1-\frac{r}{R}\right) + K_{-2}\bar{c}_2\left(1-\frac{r}{R}\right)\right\} r^2 dr = 4\pi R\,\bar{c}_1 D \tag{115}$$

and

$$4\pi\int_0^R \left\{ K_2\bar{C}_1\left(1-\frac{r}{R}\right) - K_{-2}\bar{C}_2\left(1-\frac{r}{R}\right)\right\} r^2 dr = 4\pi R\bar{C}_2 D\,.$$

We use (115) to evaluate $\bar{C}_1$ and $\bar{C}_2$. We find that

$$\left.\begin{aligned} \bar{C}_1 &= \frac{4K_1R^2(K_{-2}R^2+12D)}{144D^2+12DR^2(K_{-1}+K_2+K_{-2})+K_{-1}K_{-2}R^4}, \\ \bar{C}_2 &= \frac{4K_1K_2R^4}{144D^2+12DR^2(K_{-1}+K_2+K_{-2})+K_{-1}K_{-2}R^4}. \end{aligned}\right\} \tag{116}$$

Since the total rate of production of C_2 is given by $4\pi R\bar{C}_2 D_1$, (76) follows.

A very similar calculation can be made for a cylinder of radius h_0 and length z_0. We again assume that

$$\left.\begin{aligned} C_1(h,z) &= \bar{C}_1\left(1-\frac{h}{h_0}\right)\left(1\pm\frac{2z}{z_0}\right) \\ C_2(h,z) &= \bar{C}_2\left(1-\frac{h}{h_0}\right)\left(1\pm\frac{2z}{z_0}\right). \end{aligned}\right\} \tag{117}$$

Equating the total rate of production of C_1 and C_2 with their outflows gives us

$$2\pi\int_{-z_0/2}^{z_0/2} dz\int_0^{h_0}\left(K_1+[K_{-2}\bar{C}_2-(K_{-1}+K_2)\bar{C}_1] \times\left[1-\frac{h}{h_0}\right]\left[1\pm\frac{2z}{z_0}\right] h\,dh = \frac{4\pi D h_0^2\bar{C}_1}{z_0}+\pi\bar{C}_1 z_0 D\right) \tag{118}$$

and

$$2\pi\int_{-z_0/2}^{z_0/2} dz\int_0^{h_0}[K_2\bar{C}_1-K_{-2}\bar{C}_2]\left(1-\frac{h}{h_0}\right)\left(1\pm\frac{2z}{z_0}\right)h\,dh = \frac{4\pi D h_0^2\bar{C}_2}{z_0}+\pi\bar{C}_2 z_0 D\,. \tag{119}$$

From (118) and (119) $\bar{C}_1$ and $\bar{C}_2$ can be determined. Since

$$W_2 = \pi \bar{C}_2 D \frac{(4h_0^2 + z_0^2)}{z_0},$$

(77) follows from the determination of $\bar{C}_2$.

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RECEIVED 4–10–54

NOTE: It was called to the author's attention during proofreading that there is only one stable point in Figure 6. However, if the following sets of numbers are used, there are two stable and two unstable points (the graphs are very similar): $k_1 = 21$, $k_2 = 20$, $k_3 = 1$, $k_4 = 6$, $\sigma_1 = 0.6$, and $\sigma_1 = 1$.