

## **Systems Approach to the Study of Drug Transport Across Membranes Using Suspension Cultures of Mammalian Cells**

### **V. Simultaneous Passive Transport and Biosynthesis**

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*(Received 23 April 1973, and in revised form 20 January 1976)*

A physical model is described for the simultaneous enzymatic bioconversion of a nonelectrolyte solute and the passive transport of both the solute and product of the enzymatic reaction out of cells in culture suspension. The plasma membrane is assumed to be the rate-determining transport barrier. This model provides the basis for the experimental design and analysis of the Michaelis-Menten kinetic parameters of simple enzymatic reactions *in situ*, the phenomenological transport parameters and other factors. The primary set of differential equations describing the quasi-steady state rate of change in the concentration of the solute and product within the cell due to enzyme reaction and transport are given. These are nonlinear and must be solved by numerical methods. However, analytical mathematical expressions have been derived for various cases in the limit when the rate of enzymatic reaction is first or zero order.

### **1. Introduction**

The ultimate goal of bioavailability studies is to understand the mechanisms of drug action in the whole organism. Metabolism is an important component in this larger problem because the rates of synthesis (from a prodrug) or degradation of an active drug species affects the time course of the pharmacological response. Unfortunately, the study of the time course of the drug and its metabolites in the whole organism does not yield some of the finer mechanistic detail that might be useful in designing a more effective drug or a more efficient drug delivery system. The use of purified enzymes and tissue homogenates in *in vitro* systems does yield a wealth of information at the

mechanistic level, however, one is never quite sure how many artifacts were introduced by the experimenter. The use of intact human cells in culture suspension, while not completely free of possible artifacts, offers a number of distinct advantages over the use of the latter *in vitro* systems.

In such a system, the transport of the drug molecule through the plasma membrane and the intracellular rates of enzyme catalysis can be studied and correlated to a cellular pharmacological response. It is the determination of the intracellular rates of metabolism that are especially interesting since in the cell culture system they are modulated by a set of physical parameters native to the intact viable cell and not an artificial set imposed by the experimenter.

Many times in the past (in *in vivo* and *in vitro* studies) the metabolic rate of synthesis of a substance has been confused with the rate of incorporation of a given precursor into the substance in question (Dietschy, 1970; Plagemann & Erbe, 1972). If one is intent on determining the rate constants for the metabolism of a drug, Dietschy (1970) has pointed out the importance of recognizing such factors as (1) the rate of penetration of a drug through the plasma membrane, (2) the existence of intracellular pools of the drug, and (3) the existence of intermediate compounds between the drug and its final product. Of course, if the drug is not endogenous, the situation is greatly simplified. A number of authors have qualitatively treated the problem of simultaneous transport and biosynthesis.

Plagemann & Erbe (1972) have cited several authors (Smets, 1969; Fuchs & Kohn, 1971) who found that the rate of thymidine incorporation into deoxyribonucleic acid (DNA) in cells does not accurately reflect the rate of DNA synthesis. One possible reason for this discrepancy is that the rate of uptake of the precursor into the cell is rate limiting for its incorporation into the macromolecule in question (Plagemann & Roth, 1969; Plagemann, 1971; Plagemann & Erbe, 1972). In the case of Novikoff hepatoma cells in suspension, both a diffusional and a Michaelis-Menten transport system appear to be possible mechanisms which govern uptake. When the concentrations of thymidine are low, the Michaelis-Menten transport system is the relevant mechanism which limits the rate of incorporation of thymidine into DNA. Plagemann & Estensen (1972) have found that cytochalasin B affects this transport system but does not affect the rate of DNA and ribonucleic acid (RNA) synthesis from thymidine and uridine respectively. Jenden & Bassham (1968) and Duckworth (1970) have examined the simultaneous transport of precursors and the biosynthesis of macromolecules in isolated chloroplasts and *E. coli B*, respectively, in suspension systems. The authors, however, did not attempt to determine the limiting step in the incorporation of the precursors into the macromolecule. For a more

quantitative evaluation of the data, the problem of simultaneous transport and biosynthesis must be treated mathematically.

Any mathematical treatment of the cell which allows for the transport of the substrate and the enzymatic reaction of the substrate inside the cell must be of sufficient symmetry that the mathematical analysis of the system is tractable; a spherically symmetric model of the cell is the simplest and yet experimentally one of the most accessible geometrical configurations that has been studied thus far.

Rashevsky (1948) used Fick's law for diffusion to model the metabolizing spherical symmetric cell assuming steady state. Using this assumption, he was able to derive an expression for the total diffusional resistance of the cell and the radial concentration profiles of the substrate inside and outside of the cell. Hearon (1953) generalized Rashevsky's treatment to the situation when the rate of metabolism need not be the same at every point in the cell. Since actual Michaelis-Menten kinetics will be bounded by zero order (Rashevsky, 1948) and first order (Hearon, 1953) metabolism kinetics, Blum & Jenden (1957) expanded the source factor for the Michaelis-Menten rate of metabolism in a Taylor series in order to treat the intermediate range of concentrations. Because of experimental difficulties, earlier workers such as Best (1960) preferred to measure the change in the substrate level in the external fluid and not in the cells for an uptake experiment. Clearly the cell fraction would be more sensitive, analytically, for this purpose if one can remove the cells from the suspension and quench the metabolism. When the partitioning of the substrate into the cells is very small compared to the fluid fraction, it is mandatory to assay the cell fraction. The major assumption that Best (1960) and Blum & Jenden (1957) made in deriving expressions for the evaluation of the diffusional and biosynthetic parameters from the experimental data was that the rate of conversion of the substrate by the enzyme was the same as the rate of penetration of the substrate into the cell. While this was true for the substrates that Best (1960) studied, it is entirely conceivable that some substrates are metabolized slower or faster than they are transported into the cell's interior milieu. Thus while the steady state assumption leads to mathematical simplicity it is possible that it cannot be justified for every substrate. The quasi-steady state formulation on the other hand, which follows, is not restricted in this way.

In the first paper of this series, physical models for the diffusional transport of nonelectrolyte solutes across membranes of cells in suspension were introduced (Ho, Turi, Shipman & Higuchi, 1972). Following the quasi-steady state flux of drug across the plasma membrane, the distribution of drug in the cell interior was postulated to follow one of three principal models: (a) nonsteady state distribution in the heterogeneous cell interior, (b) rapid

(instantaneous) equilibration in the heterogeneous cell interior and (c) rapid (instantaneous) equilibration in the aqueous environment with slow simultaneous permeation of drug into the cytoplasmic bodies and nucleus.

Subsequently, experimental baseline studies on the uptake and release of various sterols and cardiac glycosides using Burkitt lymphoma cells in culture suspension at pH 7.3 under iso-osmotic conditions have been successful in gaining a physicochemical and quantitative understanding of the factors involved in the transport of these solutes (Turi, Higuchi, Shipman & Ho, 1972; Turi, 1972; Turi, Higuchi, Ho & Shipman, 1975*b*). The second of the above three models was the one found to be in good agreement with the above experimental data, i.e. the rate-determining barrier to the passive transport of the sterols and cardiac glycosides appear to be the plasma membrane and not the stagnant aqueous layer about the cell nor the membranes of the cytoplasmic bodies and nucleus within the cell. Furthermore, variations of this model were derived to include the simultaneous binding of the drug to serum in the external media and to the outermost surface of the cell membrane, and the transport of the unbound drug into the cell (Turi, Ho, Higuchi & Shipman, 1975*a*).

Next in the order of complexity of drug transport models across cell membranes, the simultaneous passive transport and bioconversion of a solute is presented. Again, the model is mathematically described in such a manner so that meaningful baseline experiments can be designed, and physical transport and chemical kinetic parameters are experimentally accessible.

## 2. General Description of the Model

A physical chemical model is developed which simultaneously accounts for solute diffusion, biosynthesis, and adsorption onto intracellular organelles in culture suspensions of mammalian cells. Figure 1 is a schematic diagram of a cell with the solute interactions indicated.

The diffusion of the substrate  $D_i$  and the product  $C_i$  out of the cell (Ho, Turi, Shipman & Higuchi, 1972) is given by the following expressions

$$-\frac{dD_i}{dt} = \frac{3P_D}{a} \left( \frac{D_i}{K_D} - D_0 \right) \quad (1)$$

$$-\frac{dC_i}{dt} = \frac{3P_C}{a} \left( \frac{C_i}{K_C} - C_0 \right) \quad (2)$$

where  $D_i$  and  $C_i$  are the total concentration of  $D$  and  $C$ , the substrate and

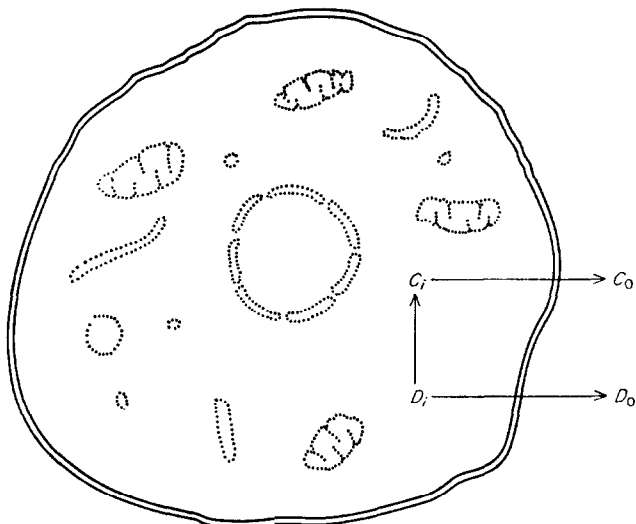


FIG. 1. Schematic model for simultaneous biosynthesis and transport. Substrate  $D_i$  is enzymatically converted to product  $C_i$  and both are transported passively out of the cell.

product respectively, inside the cell;  $D_o$  and  $C_o$  are the total concentrations of  $D$  and  $C$ , respectively, in the bulk aqueous phase outside of the cell;  $P_D$  and  $P_C$  are the permeability coefficients of  $D$  and  $C$  respectively,  $K_D$  and  $K_C$  are their partition coefficients,†  $a$  is the radius of the cell, and the factor  $3/a$  represents the surface area to volume ratio per cell taken as a sphere.

Equations (1) and (2) are based on three important assumptions. First, it is assumed that there is a rapid equilibrium within the cell's heterogeneous interior with respect to the compounds to be studied so that the unbound fraction of these compounds is homogeneously distributed throughout the interior of the cell at all times. Next, it is assumed that the permeability of the plasma membrane is the rate determining step for the diffusion of the compounds out of the cell. Only nonelectrolytes are considered and no active transport is assumed to take place. The unbound solute is assumed to be the principal membrane-permeable specie. Experimental agreement with the model seems to indicate that these are valid assumptions for some sterols and cardiac glycosides (Turi, Higuchi, Shipman & Ho, 1972; and Turi, 1972). The final assumption is that the flux across the plasma membrane is a

† In the situation where there is serum binding of  $D$  and  $C$  in the bulk aqueous phase outside of the cell,  $P$  and  $K$  become effective parameters (Turi, Higuchi, Shipman and Ho, 1972).

quasi-steady state flux. This means that the concentration gradient across the plasma membrane is the same as that which would occur if steady-state were maintained. This is usually the case with thin membranes like the plasma membrane.

The biosynthesis of  $C_i$  from  $D_i$  is assumed to follow the classical one substrate Michaelis-Menten kinetics given by

$$-\frac{dD_i}{dt} = \frac{v_{\max}D_i}{K_m + D_i} \quad (3)$$

Although the biosynthesis of  $C_i$  from  $D_i$  may, in fact, follow a more complex mechanism, it is assumed that it follows the same form of the above rate equation. Two other important assumptions must be made with regard to the applicability of equation (3) to the intracellular milieu. These assumptions are that a dynamic quasi-steady state situation exists within the cell with respect to the substrate and product, and that the possible allosteric transformations and regulatory interactions within the cell are such so as to maintain a constant specific activity of enzyme with respect to the substrate concentration.

As a further simplifying approximation, equation (3) assumes that the total solute inside the cell,  $D_i$ , is the substrate for enzymatic conversion, and therefore, it does not discriminate as to whether the relevant substrate is the free or a specific protein-bound solute among the other bound solute species. In fact, one would be experimentally hard pressed to elucidate such details. In the event that the unbound solute,  $D_{i,f}$ , is the relevant substrate, then equation (3) should be rewritten as

$$-\frac{dD_{i,f}}{dt} = \frac{v_{\max}D_{i,f}}{K_m + D_{i,f}} \quad (4)$$

Here the mass balance can be expressed by

$$D_i = D_{i,f} + \sum_j n_j k_{ad,j} D_{i,f} \quad (5)$$

where  $k_{ad,j}$  is the linear adsorption constant for the  $j$ th kind of adsorbent (e.g. a mitochondria, nuclear membrane, endoplasmic reticulum, etc.) assuming a Langmuirian isotherm in the linear region with respect to solute concentration, and where  $n_j$  is the number of adsorbents of the  $j$ th kind. Consequently, in terms of  $D_i$ , equation (4) is equivalent to

$$-\frac{dD_i}{dt} = \frac{v_{\max}D_i}{K_m + D_i \left(1 + \sum_j n_j k_{ad,j}\right)} \quad (6)$$

Coupling equations (1) and (2) to (3) for the simultaneous diffusion of the compounds out of the cell and for the biosynthesis of  $C$  from  $D$ , we have

$$-\frac{dD_i}{dt} = \frac{3P_D}{a} \left( \frac{D_i}{K_D} - D_0 \right) + \frac{v_{\max} D_i}{K_m + D_i} \quad (7)$$

$$-\frac{dC_i}{dt} = \frac{3P_C}{a} \left( \frac{C_i}{K_C} - C_0 \right) - \frac{v_{\max} D_i}{K_m + D_i} \quad (8)$$

The mass balance equations are:

$$T_D = D_0 V_0 + n V_i D_i \quad (9)$$

$$T_C = C_0 V_0 + n V_i C_i \quad (10)$$

$$T = T_C + T_D \quad (11)$$

where  $V_i$  is the cell volume of an essentially monodispersed cell size distribution,  $n$  is the number of cells,  $V_0$  is the external bulk aqueous phase volume, and  $T_D$  and  $T_C$  are the total amount of  $D$  and  $C$ , respectively, in the system. If  $T$  is conserved, then an important relationship for tracer studies is that the decrease in the total concentration of  $D$  in the closed system with time is related to the bioconversion of  $D_i$  to  $C_i$  within the cell; i.e.,

$$-\frac{1}{V} \frac{dT_D}{dt} = \frac{v_{\max} D_i}{K_m + D_i} \quad (12)$$

where  $V = nV_i + V_0$ .

The initial boundary conditions for this model are:

$$C_i = C_i(0)$$

$$D_i = D_i(0)$$

$$T_D = T_D(0)$$

$$T_C = T_C(0)$$

$$C_0(0) = 0$$

$$D_0(0) = 0$$

Equations (7), (8), and (12) form a set of nonlinear differential equations describing the quasi-steady state rate of change in the concentration of the drug and product within the cell due to enzyme reaction and transport. They can be solved by numerical methods. In order to gain a more explicit and helpful insight into the immediate treatment and interpretation of experimental data, we seek approximate, but analytic, solutions to the above primary set of differential equations.

### 3. Case I—Linear Approximation to Biosynthesis

Consider the situation when  $C_i$  and  $D_i$  are free to diffuse out of the cell, and the biosynthesis of  $C_i$  from  $D_i$  occurs in the limit  $K_m \gg D_i$ . Consequently, equations (7), (8), and (12) become

$$-\frac{dD_i}{dt} = \frac{3P_D}{a} \left( \frac{D_i}{K_D} - D_0 \right) + \frac{v_{\max}}{K_m} D_i \quad (13)$$

$$-\frac{dC_i}{dt} = \frac{3P_C}{a} \left( \frac{C_i}{K_C} - C_0 \right) - \frac{v_{\max}}{K_m} D_i \quad (14)$$

$$\frac{1}{V} \frac{dT_D}{dt} = -\frac{v_{\max}}{K_m} D_i \quad (15)$$

As shown in Appendix A, the solutions are:

$$T_D = C_1 e^{r_1 t} + C_2 e^{r_2 t} \quad (16)$$

$$D_i = -\frac{K_m}{v_{\max} V} [C_1 r_1 e^{r_1 t} + C_2 r_2 e^{r_2 t}] \quad (17)$$

and

$$C_i = -C_1 \frac{\delta_1}{(r_1 + \gamma)} e^{r_1 t} - C_2 \frac{\delta_2}{(r_2 + \gamma)} e^{r_2 t} + C_3 e^{-\gamma t} + \frac{\varepsilon}{\gamma} \quad (18)$$

where

$$r_{1,2} = -\frac{\beta}{2} \pm \sqrt{\beta^2/4 - \alpha} \quad (19)$$

$$\beta = \frac{v_{\max}}{K_m} + \frac{3P_0}{a} \left[ \frac{nV_i}{V_0} + \frac{1}{K_D} \right] \quad (20)$$

$$\alpha = \frac{3P_D V}{aV_0} \cdot \frac{v_{\max}}{K_m} \quad (21)$$

The constants  $\varepsilon$ ,  $\gamma$ ,  $\delta_1$  and  $\delta_2$  are defined in Appendix A. The constants  $C_1$ ,  $C_2$ , and  $C_3$  can be determined from the initial conditions and are given by equations (A13), (A14) and (A15) in Appendix A. It is noted that the constants  $\alpha$ ,  $\beta$ ,  $\varepsilon$ ,  $\gamma$ ,  $C_1$ ,  $C_2$  and  $C_3$  are positive quantities. In contrast,  $r_1$  and  $r_2$  are negative quantities such that  $|r_2| > |r_1|$ .

The fundamental kinetic parameter  $v_{\max}/K_m$  can be determined from equations (19), (20), and (21) once  $r_1$  is determined, and it is given by the expression

$$-\frac{v_{\max}}{K_m} = \frac{r_1 \left[ r_1 + \frac{3P_0}{a} \left( \frac{nV_i}{V_0} + \frac{1}{K_D} \right) \right]}{\left[ r_1 + \frac{1}{V} \frac{3P_D}{aV_0} \right]} \quad (22)$$



The permeability coefficients of the plasma membrane,  $P_D$  and  $P_C$ , and the partition coefficients,  $K_D$  and  $K_C$ , can be determined from independent experiments with solutes  $D$  and  $C$ . For example, experiments involving the release of solute  $D$  from cells or its uptake by the cells in which various experimental conditions are imposed to prevent bioconversion reactions would result in determining  $P_D$  and  $K_D$  (Turi, Higuchi, Shipman & Ho, 1972; Turi, Higuchi, Ho & Shipman, 1975*b*).

#### 4. Case II—Taylor Series Expansion of the Biosynthetic Factor

The nonlinear factor in equation (3) may be expanded in a Taylor series about  $D_i = D_i(0)$  at  $t = 0$ . To the first order

$$\frac{v_{\max} D_i}{K_m + D_i} = \frac{v_{\max} D_i(0)}{K_m + D_i(0)} + [D_i - D_i(0)] \cdot \frac{d}{dD_i} \left[ \frac{v_{\max} D_i}{K_m + D_i} \right] \Bigg|_{D_i = D_i(0)} \quad (23)$$

Therefore, equation (3) becomes

$$-\frac{dD_i}{dt} = \omega + \omega' D_i \quad (24)$$

where

$$\omega = \frac{v_{\max} D_i(0)^2}{[D_i(0) + K_m]^2}$$

$$\omega' = \frac{v_{\max} K_m}{[D_i(0) + K_m]^2}$$

so that equations (7), (8) and (12) become

$$-\frac{dD_i}{dt} = \frac{3P_D}{a} \left( \frac{D_i}{K_C} - D_0 \right) + (\omega + \omega' D_i) \quad (25)$$

$$-\frac{dC_i}{dt} = \frac{3P_C}{a} \left( \frac{C_i}{K_C} - C_0 \right) - (\omega + \omega' D_i) \quad (26)$$

$$\frac{1}{V} \frac{dT_D}{dt} = -(\omega + \omega' D_i) \quad (27)$$

Equations (25), (26) and (27) along with the mass balance equations describe the system. The solutions for  $T_D$  and  $D_i$  are:

$$T_D = B_1 e^{s_1 t} + B_2 e^{s_2 t} + \frac{k''}{k'} \quad (28)$$

$$D_i = \frac{-1}{\omega' V} (B_1 s_1 e^{s_1 t} + B_2 s_2 e^{s_2 t}) - \frac{\omega}{\omega'} \quad (29)$$

where

$$s_{1,2} = -\frac{k}{2} \pm \sqrt{k^2/4 - k'}$$

$$k = \frac{3P_D}{a} \left[ \frac{nV_i}{V_0} + \frac{1}{K_D} \right] + \omega'$$

$$k' = \frac{3P_D V \omega'}{a V_0} \quad k'' = -\omega V \left[ \frac{3P_D}{a} \left( \frac{nV_i}{V_0} + \frac{1}{K_D} \right) \right]$$

$B_1$  and  $B_2$  are determined from the initial conditions and are described in Appendix B. It is noted that the constants  $\omega$ ,  $\omega'$ ,  $k$ ,  $k'$ ,  $B_1$  and  $B_2$  are positive quantities and the constants  $k''$ ,  $S_1$  and  $S_2$  are negative quantities such that  $|S_2| > |S_1|$ .

Two remarks are in order about the comparison of case I and case II. In case I, the limit of  $K_m \gg D_i$  allowed us to approximate equation (3) and the equations dependent on (3) by the term  $v_{\max} D_i / K_m$ . In case II equation (3) is approximated by  $\omega + \omega' D_i$ . But, in the limit as  $D_i(0)$  tends to zero, it follows that  $\omega$  and  $\omega'$  tend to zero and  $v_{\max} / K_m$ , respectively, and all of the constants of case II, such as  $s_{1,2}$ ,  $k$ ,  $k'$ , etc., tend to their corresponding values as defined in case I.

Finally, one notes that

$$\omega' = \frac{-s_1 \left[ s_1 + \frac{3P_D}{a} \left( \frac{nV_i}{V_0} + \frac{1}{K_D} \right) \right]}{s_1 + \frac{3P_D V}{a V_0}} \quad (30)$$

Since  $\omega'$  by equation (24) is a function of  $v_{\max}$ ,  $K_m$  and  $D_i(0)$ , the theory implies that equation (30) determines  $v_{\max}$  and  $K_m$  independent of equation (22), given  $D_i(0)$  and  $s_1$ .

### 5. Case III—Enzyme Saturation

In this case, when  $D_i \gg K_m$ , the substrate is in great excess of the enzyme concentration and equation (3) is approximated by  $v_{\max}$ . Thus, equations (7), (8) and (12) become

$$-\frac{dD_i}{dt} = \frac{3P_D}{a} \left( \frac{D_i}{K_D} - D_0 \right) + v_{\max} \quad (31)$$

$$-\frac{dC_i}{dt} = \frac{3P_C}{a} \left( \frac{C_i}{K_C} - C_0 \right) - v_{\max} \quad (32)$$

$$\frac{1}{V} \frac{dT_D}{dt} = -v_{\max} \quad (33)$$

The solutions to this system of equations are:

$$T_D = -v_{\max}Vt + T_D(0) \quad (34)$$

$$D_i = A_1 e^{-\rho t} + \frac{v_{\max}\mu V}{\rho^2} (1 - \rho t) + \frac{1}{\rho} [\mu T_D(0) - v_{\max}] \quad (35)$$

$$C_i = A_2 e^{-\psi t} + \frac{1}{\psi} [v_{\max} + \phi(T - T_D(0))] + \frac{\phi}{\psi^2} v_{\max} V (\psi t - 1) \quad (36)$$

where the constants  $\rho$ ,  $\mu$ ,  $\psi$ , and  $\phi$  are defined in Appendix C and  $A_1$  and  $A_2$  are determined from the initial conditions and are given by (C6) and (C7).

## APPENDIX A

### Case I—Linear Approximation to Biosynthesis

The elimination of  $D_0$  in equation (13) with (9) gives

$$\frac{dD_i}{dt} = \frac{3P_D}{a} \left[ \frac{T_D}{V_0} - \left( \frac{nV_i}{V_0} + \frac{1}{K_D} \right) D_i \right] - \frac{v_{\max}}{K_m} D_i \quad (A1)$$

Using equation (15), we can express equation (A1) accordingly:

$$\frac{d^2 T_D}{dt^2} + \beta \frac{dT_D}{dt} + \alpha T_D = 0 \quad (A2)$$

where

$$\beta = \frac{v_{\max}}{K_m} + \frac{3P_D}{a} \left[ \frac{nV_i}{V_0} + \frac{1}{K_D} \right]$$

$$\alpha = \frac{3P_D}{aV_0} \frac{Vv_{\max}}{K_m}$$

The solution is

$$T_D = C_1 e^{r_1 t} + C_2 e^{r_2 t} \quad (A3)$$

where

$$r_{1,2} = -\frac{\beta}{2} \pm \sqrt{\beta^2/4 - \alpha}$$

and  $C_1$  and  $C_2$  are constants to be determined by the initial conditions.

After differentiating equation (A3) and substituting into (15), we get

$$D_i = -\frac{K_m}{v_{\max}V} [C_1 r_1 e^{r_1 t} + C_2 r_2 e^{r_2 t}] \quad (A4)$$

To find  $C_i$  we start with equation (14) and eliminate  $C_0$  and  $T_c$  by using equations (10) and (11); thus,

$$\frac{dC_i}{dt} + \frac{3P_c}{a} \left( \frac{nV_i}{V_0} + \frac{1}{K_c} \right) C_i = \frac{3P_c}{aV_0} (T - T_D) + \frac{v_{\max}}{K_m} D_i \quad (\text{A5})$$

whereupon the substitution of equations (A3) and (A4) for  $T_D$  and  $D_i$ , respectively, gives

$$\frac{dC_i}{dt} + \gamma C_i = \varepsilon - C_1 \delta_1 e^{r_1 t} - C_2 \delta_2 e^{r_2 t} \quad (\text{A6})$$

where

$$\begin{aligned} \gamma &= \frac{3P_c}{a} \left( \frac{nV_i}{V_0} + \frac{1}{K_c} \right) \\ \varepsilon &= \frac{3P_c T}{aV_0} \\ \delta_1 &= \frac{3P_c}{aV_0} + \frac{r_1}{V} \\ \delta_2 &= \frac{3P_c}{aV_0} + \frac{r_2}{V} . \end{aligned}$$

Equation (A6) is a nonhomogeneous linear differential equation with constant coefficients. The solution to the associated homogeneous differential equation is  $C_3 \exp(-\gamma t)$  and the particular solution is assumed to be of the form  $v_3(t) \exp(-\gamma t)$ . The general solution is expressed by

$$C_i = C_3 e^{-\gamma t} + v_3 e^{-\gamma t} \quad (\text{A7})$$

and

$$\frac{dC_i}{dt} = -C_3 \gamma e^{-\gamma t} - v_3 \gamma e^{-\gamma t} + e^{-\gamma t} \cdot \frac{dv_3}{dt} \quad (\text{A8})$$

After combining equations (A6), (A7) and (A8) and then integrating,

$$v_3(t) = \frac{\varepsilon}{\gamma} e^{\gamma t} - C_1 \frac{\delta_1}{(r_1 + \gamma)} e^{(r_1 + \gamma)t} - C_2 \frac{\delta_2}{(r_2 + \gamma)} e^{(r_2 + \gamma)t} \quad (\text{A9})$$

and, consequently,

$$C_i = -C_1 \frac{\delta_1}{r_1 + \gamma} e^{r_1 t} - C_2 \frac{\delta_2}{r_2 + \gamma} e^{r_2 t} + C_3 e^{-\gamma t} + \frac{\varepsilon}{\gamma} . \quad (\text{A10})$$

Equations (A3), (A4) and (A10) form the solution set to the system of differential equations for this case. The constants  $C_1$ ,  $C_2$ ,  $C_3$  can be

determined from the initial conditions:

$$\begin{aligned} C_i &= C_i(0) \\ D_i &= D_i(0) \\ T_D &= T_D(0) \end{aligned}$$

Thus,

$$C_1 = \frac{v_{\max}V}{K_m(r_2-r_1)} D_i(0) + \frac{r_2}{(r_2-r_1)} T_D(0) \tag{A11}$$

$$C_2 = \frac{v_{\max}V}{K_m(r_1-r_2)} D_i(0) + \frac{r_1}{(r_1-r_2)} T_D(0) \tag{A12}$$

$$\begin{aligned} C_3 = \frac{v_{\max}V}{K_m(r_1-r_2)} & \left( \frac{\delta_2}{r_2+\gamma} - \frac{\delta_1}{r_1+\gamma} \right) D_i(0) + \frac{1}{(r_1-r_2)} \times \\ & \times \left( \frac{\delta_2 r_1}{(r_2+\gamma)} - \frac{\delta_1 r_2}{(r_1+\gamma)} \right) T_D(0) + C_i(0) - \frac{\varepsilon}{\gamma}. \end{aligned} \tag{A13}$$

APPENDIX B

Case II—Taylor Series Expansion of Equations (3)

The derivation for this case exactly parallels that of case I. Substituting equations (9) and (27) into (25), we obtain

$$\frac{d^2 T_D}{dt^2} + k \frac{dT_D}{dt} + k' T_D = k'' \tag{B1}$$

where

$$\begin{aligned} k &= \frac{3P_D}{a} \left[ \frac{nV_i}{V_0} + \frac{1}{K_D} \right] + \omega' \\ k' &= \frac{3P_D \omega' V}{aV_0} \\ k'' &= -\omega V \left\{ \frac{3P_D}{a} \left[ \frac{nV_i}{V_0} + \frac{1}{K_D} \right] \right\} \end{aligned}$$

The solution to equation (B1) is

$$T_D = B_1 e^{s_1 t} + B_2 e^{s_2 t} + \frac{k''}{k'} \tag{B2}$$

where

$$s_{1,2} = -\frac{k}{2} \pm \sqrt{k^2/4 - k'}$$

The combination of equation (27) and the derivative of (B2) leads to

$$D_i = -\frac{1}{\omega'V}(B_1s_1 e^{s_1t} + B_2s_2 e^{s_2t}) - \frac{\omega}{\omega'} \quad (\text{B3})$$

Using the initial conditions, we find that  $B_1$  and  $B_2$  are

$$B_1 = \frac{[T_D(0) - k''/k']s_2 + [\omega/\omega' + D_i(0)]\omega'V}{s_2 - s_1} \quad (\text{B4})$$

$$B_2 = \frac{[T_D(0) - k''/k']s_1 + [\omega/\omega' + D_i(0)]\omega'V}{s_1 - s_2} \quad (\text{B5})$$

## APPENDIX C

### Case III—Enzyme Saturation

Equation (33) can be directly integrated to give

$$T_D = -v_{\max}Vt + T_D(0). \quad (\text{C1})$$

Substituting in equations (9) and (C1) into (31) gives

$$\frac{dD_i}{dt} + \rho D_i = \mu T_D(0) - v_{\max}(\mu Vt + 1) \quad (\text{C2})$$

where

$$\rho = \frac{3P_D}{a} \left( \frac{nV_i}{V_0} + \frac{1}{K_D} \right)$$

$$\mu = \frac{3P_D}{aV_0}.$$

The solution to (C2) can be found by the method of variation of parameters as in Appendix A. The solution is

$$D_i = A_1 e^{-\rho t} + \frac{v_{\max}\mu V}{\rho^2} (1 - \rho t) + \frac{1}{\rho} [\mu T_D(0) - v_{\max}]. \quad (\text{C3})$$

Similarly equation (32) becomes

$$\frac{dC_i}{dt} + \psi C_i = v_{\max} + \phi [T - T_D(0)] + \phi v_{\max} Vt \quad (\text{C4})$$

$$\psi = \frac{3P_C}{a} \left( \frac{nV_i}{V_0} + \frac{1}{K_C} \right)$$

$$\phi = \frac{3P_C}{aV_0}$$

and the solution to (C4) is

$$C_i = A_2 e^{-\psi t} + \frac{1}{\psi} \{v_{\max} + \phi[T - T_D(0)]\} + \frac{\phi}{\psi^2} v_{\max} V(\psi t - 1) \quad (\text{C5})$$

and  $A_1$  and  $A_2$  determined from the initial conditions of are:

$$A_1 = D_i(0) + \frac{1}{\rho} v_{\max} - \mu T_D(0) - \frac{v_{\max} \mu V}{\rho^2} \quad (\text{C6})$$

$$A_2 = C_i(0) - \frac{1}{\psi} \{v_{\max} + \phi[T - T_D(0)]\} + \frac{v_{\max} \phi V}{\psi^2} \quad (\text{C7})$$

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