Systems Approach to Study of Solute Transport across Membranes Using Suspension Cultures of Mammalian Cells III: Steady-State Diffusion Models

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Abstract □ General physical models are derived for the diffusional transport of drugs across membranes of mammalian cells in culture suspension. These models represent different sets of possible physical processes taking place during the transport of a drug molecule. Once the diffusing species reaches the cell barrier, it may gain entrance to the cell kinetically by one of the principal quasi-steady-state mechanisms, all of which assume the cell membrane to be an integral part of the total barrier.

Keyphrases □ Transport mechanisms—eight steady-state diffusion models derived and discussed, membranes using suspension cultures of mammalian cells □ Diffusion models—eight steady-state models derived and discussed, membranes using suspension cultures of mammalian cells □ Drug transport—studied using systems approach, across membranes using suspension cultures of mammalian cells, eight steady-state diffusion models derived and discussed □ Solute binding—systems approach, drug transport across membranes using suspension cultures of mammalian cells, eight steady-state diffusion models derived and discussed

In recent years, considerable effort has been directed toward the understanding of the mechanism of drug transport across membranes. Numerous experimental systems including cell cultures have been used to study membrane transport. It is felt that these cell systems provide the means to quantify drug metabolism, distribution, and bioavailability at the site of drug action.

Requisites for quantitative, mechanistic approaches to scientific understanding are the simultaneous development of sound, theoretical physical models and the establishment of experimental systems that are accessible to rigorous analysis by physicochemical techniques.

This paper discusses some general physical models for the diffusional transport of drugs across membranes of cells in culture suspension; they represent extensions of some earlier models (1–3). These models provide the quantitative interrelationships among the variables important to the transport of drug molecules across cell membranes. They also provide the basis for the design and analysis of experiments and the foundation for development of new physical models. The use of these physical models with the experimental data is intended to describe the nature of the transport barriers, the kinds of species transported, and whether, where, and how much solute binding occurs. These models assume that the aqueous diffusion layer has a negligible effect on the transport rate.

PHYSICAL MODELS

The eight physical models, each designed to represent different sets of possible physical processes, are:

MODEL 1. Rapid equilibration in the heterogeneous cell interior in which no binding of the drug molecule to components outside the cell occurs.

MODEL 2. Rapid equilibration in the heterogeneous cell interior in which binding of the drug molecule to components outside the cell occurs.

MODEL 3. Rapid equilibration of the drug in the heterogeneous cell interior with an instantaneous, irreversible binding of the drug molecule to the cell plasma membrane at initial time.

MODEL 4. Rapid equilibration of the drug in the heterogeneous cell interior accompanied by reversible, instantaneous binding of the drug molecule to the cell plasma membrane.

MODEL 5. Rapid equilibration of the drug in the heterogeneous cell interior accompanied by reversible, instantaneous binding of the drug molecule to the cell plasma membrane followed by the permeation of both the unbound drug and the membrane-bound drug through the plasma membrane.

MODEL 6. Simultaneous transport of two drugs, each rapidly equilibrated in the heterogeneous cell interior in which neither binds to components outside the cell.

MODEL 7. Simultaneous transport of two drugs, each rapidly equilibrated in the heterogeneous cell interior in which one binds to the plasma membrane by the conditions in Model 4.

MODEL 8. Rapid equilibration in the aqueous environment in the cell, with slow simultaneous permeation of the drug into the cytoplasmic bodies and the nucleus.

A schematic diagram of the cell used for the development of these models is shown in Fig. 1.

Model 1—In Model 1 the plasma membrane is rate limiting for the diffusion of nonionic molecules.

Assuming that adsorption is negligible, the quasi-steady-state rate of uptake of a drug by a cell can be described by:

$$\frac{dC_i}{dt} = \frac{4\pi a^2 P}{V_i}\left(C_0 - \frac{C_i}{K}\right) \qquad \text{(Eq. 1)}$$

where P is the intrinsic permeability coefficient of the plasma membrane, C_i is the total drug concentration in the cell, a is the cell radius, C_0 is the drug concentration in the external medium, and K is the intrinsic partition coefficient. In reality, K is an apparent partition or distribution coefficient encompassing all interactions and may be considered equal to the total drug bound, partitioned, unbound, or otherwise distributed in the cell at equilibrium divided by $nV_iC_{0,\infty}$, where V_i is the volume of the cell. Since $V_i = (4/3)\pi a^3$, Eq. 1 becomes:

$$\frac{dC_i}{dt} = \frac{3P}{a}\left(C_0 - \frac{C_i}{K}\right) \qquad \text{(Eq. 2)}$$

Mass balance of the drug requires that:

$$T = C_0V_0 + nV_iC_i \qquad \text{(Eq. 3)}$$

where T is the total amount of drug in the system, n is the number of cells, and V_0 is the volume of the external medium. Equation 3 is only applicable to a system with a narrow cell volume distribution, as is the case with the Burkitt lymphoma cells; otherwise, the cell volume distribution should be considered.

By solving Eq. 3 for C_0, substituting into Eq. 2, and integrating between $C_i(0) = 0$ and $C_i(t)$, the solution for Model 1 is:

$$\ln\left(1 - \frac{BC_i}{A}\right) = -\frac{3BP}{a}(t) \qquad \text{(Eq. 4)}$$

where $A = (T/V_0)$, and $B = (nV_i/V_0) + (1/K)$.

The Model 1 uptake function (UF_1) is defined by rearranging Eq. 4 to give:

$$UF_1 = -\left[\frac{a}{(3)(60B)}\right]\ln\left(1 - \frac{BC_i}{A}\right) = Pt \qquad \text{(Eq. 5)}$$

where the factor 60 is necessary for determining the permeability coefficient in units of centimeters per second from the slope of the linear regression line of the UF_1 *versus* time (in minutes) plot.

For initial rates, Eq. 4 reduces to:

$$C_i = \frac{3PT}{aV_0}(t) \qquad \text{(Eq. 6)}$$

Thus, the intrinsic permeability coefficient, P, can be obtained from the initial slope of the C_i *versus* time plot or from the plot of Eq. 5 when K is known. The intrinsic partition coefficient, K, can be experimentally obtained from the equilibrium condition, *i.e.*, when $(dC_i/dt)_{t=\infty} = 0$; thus:

$$K = \frac{C_{i,\infty}}{C_{0,\infty}} \qquad \text{(Eq. 7)}$$

The intrinsic permeability coefficients may also be determined by the nonlinear regression analysis of Eq. 4.

Model 2—This model considers any drug binding to serum used in the external medium of the cell suspension system (Fig. 2). Equations 1–4 should be modified as follows.

The total concentration of drug in the aqueous medium is:

$$C_{0,T} = C_0 + C_0^* \qquad \text{(Eq. 8)}$$

where $C_{0,T}$ is the total drug concentration in the external phase, and C_0 and C_0^* are the concentrations of unbound and bound drug in the external phase, respectively. The Langmuir adsorption isotherm is assumed:

$$C_0^* = \frac{k_2C_0}{(1/k_1) + C_0} \qquad \text{(Eq. 9)}$$

where k_1 represents the fraction of sites occupied, and k_2 indicates the maximum amount that can be adsorbed. In the limit where k_1 is very small such that $1/k_1$ is much greater than C_0:

$$C_0^* = k_1k_2C_0 \qquad \text{(Eq. 10)}$$

where k_1k_2 is the linear adsorption constant of the serum-bound drug. This condition can also be expressed as shown here in Scheme I.

$$\text{drug} + \text{serum} \overset{K_b}{\rightleftharpoons} \text{drug–serum}$$

Scheme I

Therefore, the equilibrium binding constant is:

$$K_b = \frac{C_0^*}{C_0(S)} \qquad \text{(Eq. 11)}$$

whereupon, with Eq. 8:

$$C_{0,T} = C_0(1 + k_0) \qquad \text{(Eq. 12)}$$

where k_0 is equal to $K_b(S)$ by definition, K_b is the binding constant, and (S) is the serum concentration.

After substituting Eq. 12 into Eq. 2, the following rate expression for Model 2 is obtained:

$$\frac{dC_i}{dt} = \frac{3P_e}{a}\left(C_{0,T} - \frac{C_i}{K_e}\right) \qquad \text{(Eq. 13)}$$

The effective partition coefficient is:

$$K_e = \frac{C_i}{C_0 + C_0^*} = \frac{K}{1 + k_0} = \frac{K}{1 + K_b(S)} \qquad \text{(Eq. 14)}$$

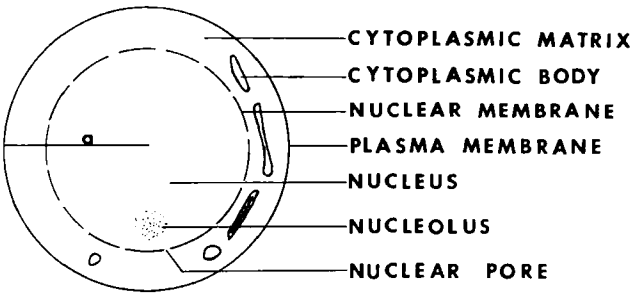

Figure 1—*Schematic description of a spherical cell of radius* a *in a viable cell culture suspension for the passive transport of nonelectrolyte drugs. In Models 1–7, the plasma membrane is the only rate-determining barrier. In Model 8, both the plasma membrane and membranes of the cytoplasmic bodies and nucleus are rate-determining barriers.*

and the effective permeability coefficient is:

$$P_e = \frac{P}{1 + k_0} = \frac{P}{1 + K_b(S)} \qquad \text{(Eq. 15)}$$

It is assumed in Eq. 15 that only the unbound drug is the membrane permeable species.

By means of Eqs. 14 and 15, the equilibrium serum–drug binding constant, K_b, can be calculated. For example, a plot of P/P_e *versus* percent of serum gives an intercept of unity and a slope equal to K_b. If the intrinsic permeability coefficient cannot be readily obtained or there is some uncertainty, then the analysis can be performed by choosing the permeability coefficient at a serum level as the reference. Thus, when choosing the permeability coefficient at 30% serum as the reference, a plot of P_{30}/P_e *versus* percent of serum gives a slope equal to $K_b/[1 + K_b(0.3)]$ and an intercept equal to $1/[1 + K_b(0.3)]$.

By using the mass balance expression:

$$T = C_{0,T}V_0 + nC_iV_i \qquad \text{(Eq. 16)}$$

the solution to Eq. 13 is:

$$\ln\left(1 - \frac{B'}{A}C_i\right) = -\frac{3P_eB'}{a}(t) \qquad \text{(Eq. 17)}$$

where $B' = (nV_i/V_0) + (1/K_e)$. The mathematical form is the same as Eq. 4 except that effective parameters are involved instead of intrinsic parameters because of the binding of the drug to the serum in the external medium.

Equations 14 and 15 are important, because they allow one to calculate the intrinsic permeability of the membrane from the effective permeability coefficients in the presence of serum, which is necessary to maintain integrity of the cells for long periods. In turn, the intrinsic permeability coefficients can be used to quantify the effect of molecular structure and functional group substituent modifications on membrane absorptivity.

Model 3—This model assumes that there is instantaneous, irreversible drug binding to the plasma membrane followed by diffu-

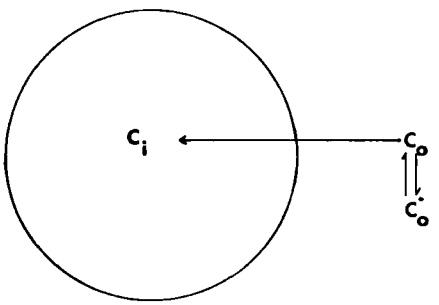

Figure 2—*Model 2: Passive transport of unbound solute across the plasma membrane with rapid distribution in the heterogeneous cell interior and reversible binding of the solute to serum and other components in the external media.*

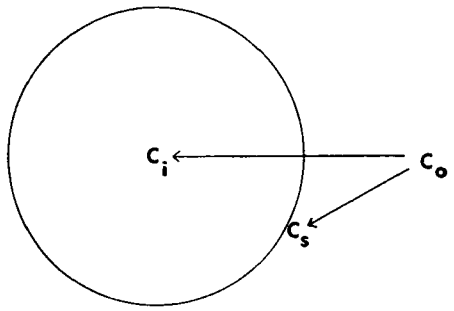

Figure 3—*Model 3: Passive transport of unbound solute across the plasma membrane with rapid distribution in the heterogeneous cell interior. There is an instantaneous, irreversible binding of the drug molecules to the external cell plasma membrane at initial time.*

sion of unbound drug molecules through the plasma membrane (Fig. 3). The following general relationships are appropriate:

$$A_c = A_i + A_s \quad \text{(Eq. 18)}$$

$$C_c = A_c/V_i \quad \text{(Eq. 19)}$$

$$C_s = A_s/V_i \quad \text{(Eq. 20)}$$

$$T = nA_c + A_0 = nC_cV_i + C_0V_0 \quad \text{(Eq. 21)}$$

where T is the total amount of drug present, A_c is the amount of drug in and on the cell, A_i is the amount inside the cell, A_s is the amount on the external surface of the cell, A_0 is the amount outside the cell, C_c is the drug concentration in and on the cell, and C_s is the drug concentration on the surface of the cell. The general mass balance relationships of Eqs. 18–21 are also applicable to Models 4 and 5.

While it is an experimental reality that C_c as a function of time is readily accessible, one encounters the problem of handling the data to assess the membrane permeability and partition coefficients clearly. This problem is resolved by the following mathematical treatment of this model.

By utilizing Eqs. 18–21:

$$C_c = C_i + C_s \quad \text{(Eq. 22)}$$

and:

$$\frac{dC_c}{dt} = \frac{dC_i}{dt} \quad \text{(Eq. 23)}$$

where $dC_s/dt = 0$ since C_s is the concentration of drug adsorbed instantaneously at initial time and irreversibly on the external surface of the plasma membrane and, consequently, is independent of the drug concentration in the external medium and time. It follows that the fundamental Eq. 2 for the quasi-steady-state rate of drug uptake by a cell can be rewritten as:

$$\frac{dC_c}{dt} = \frac{3P}{a}(X - BC_c) \quad \text{(Eq. 24)}$$

where:

$$X = \frac{T}{V_0} + \frac{C_s}{K} \quad \text{(Eq. 25)}$$

and B is as defined before. Integrating between the boundary conditions, $C_c = C_s$ at time = 0 and C_c (t):

$$\ln\left(\frac{X - BC_c}{X - BC_s}\right) = -\left(\frac{3BP}{a}\right)(t) \quad \text{(Eq. 26)}$$

which, upon rearranging, gives the Model 3 uptake function:

$$UF_3 = -\left[\frac{a}{(3)(60B)}\right]\ln\left(\frac{X - BC_c}{X - BC_s}\right) = Pt \quad \text{(Eq. 27)}$$

Before a plot of the uptake function *versus* time (in minutes)

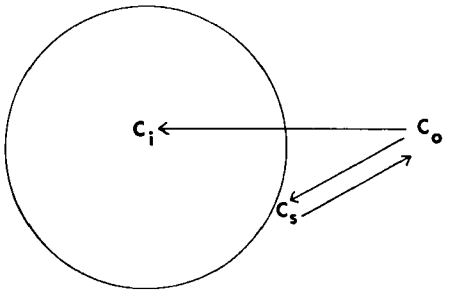

Figure 4—*Model 4: Passive transport of unbound solute across the plasma membrane with rapid distribution in the heterogeneous cell interior. There is an instantaneous, reversible binding of drug molecules to the external cell plasma membrane.*

can be made to calculate the permeability coefficient (in units of centimeters per second), the intrinsic partition coefficient must be found. Experimentally, the apparent partition coefficient, K_{ex}, is readily determined and, in turn, it is related to the intrinsic partition coefficient, K. Thus:

$$K_{ex} = \frac{C_{c,\infty}}{C_{0,\infty}} = \frac{C_{i,\infty} + C_s}{C_{0,\infty}} = K + \frac{C_s}{C_{0,\infty}} \quad \text{(Eq. 28)}$$

or:

$$K = K_{ex} - \frac{C_s}{C_{0,\infty}} \quad \text{(Eq. 29)}$$

Model 4—This model takes into account both the instantaneous and reversible drug binding to the plasma membrane and passive transport of the unbound drug molecule through the plasma membrane (Fig. 4). The concentration of drug bound to the cell surface is assumed to be proportional to the concentration of drug in the external medium:

$$C_s = K_{bm}C_0 \quad \text{(Eq. 30)}$$

where K_{bm} is the plasma membrane–drug equilibrium adsorption constant.

The uptake rate of drug into the cell interior is expressed by Eq. 2. With Eqs. 21, 22, and 30 and their derivatives, it can be shown that:

$$\frac{dC_i}{dt} = \frac{d(C_c - C_s)}{dt} = \left(1 + K_{bm}\frac{nV_i}{V_0}\right)\frac{dC_c}{dt} \quad \text{(Eq. 31)}$$

and:

$$\left(C_0 - \frac{C_i}{K}\right) = \left(1 + \frac{K_{bm}}{K}\right)\frac{T}{V_0} - \left[\left(1 + \frac{K_{bm}}{K}\right)\frac{nV_i}{V_0} + \frac{1}{K}\right]C_c \quad \text{(Eq. 32)}$$

Consequently, Eq. 2 becomes:

$$\frac{dC_c}{dt} = \frac{3P}{a(1 + K_{bm}E)}[(F - GC_c)] \quad \text{(Eq. 33)}$$

and its solution is:

$$\ln\left[\frac{F - GC_c}{F - GC_{c,0}}\right] = -\frac{3GP}{a(1 + K_{bm}E)}(t) \quad \text{(Eq. 34)}$$

where:

$$F = \left(1 + \frac{K_{bm}}{K}\right)\frac{T}{V_0} \quad \text{(Eq. 35)}$$

$$G = \left(1 + \frac{K_{bm}}{K}\right)\frac{nV_i}{V_0} + \frac{1}{K} \quad \text{(Eq. 36)}$$

$$E = \frac{nV_i}{V_0} \quad \text{(Eq. 37)}$$

and $C_{c,0}$ is the cell concentration of the drug at zero time, which is

equal to the initial surface-adsorbed drug concentration. The Model 4 uptake function is:

$$UF_4 = -\left[\frac{a(1 + K_{bm}E)}{(3)(60G)}\right]\ln\left[\frac{F - GC_c}{F - GC_{c,0}}\right] = Pt \quad \text{(Eq. 38)}$$

The drug–membrane adsorption constant may be found with the aid of Eqs. 20, 21, and 30 and from the extrapolation of a A_c *versus* t plot to $t = 0$ to get $A_{s,0}$:

$$K_{bm} = \frac{nA_{s,0}/V_i}{A_0/V_0} = \frac{nA_{s,0}}{(V_i/V_0)(T - nA_{s,0})} \quad \text{(Eq. 39)}$$

The apparent partition coefficient is determined from the equilibrium transport conditions; thus:

$$K_{ex} = \frac{C_{c,\infty}}{C_{0,\infty}} = \frac{C_{i,\infty} + K_{bm}C_{0,\infty}}{C_{0,\infty}} \quad \text{(Eq. 40)}$$

and, subsequently:

$$K = K_{ex} - K_{bm} \quad \text{(Eq. 41)}$$

Model 5—This model assumes that there is instantaneous, reversible drug binding to the plasma membrane and diffusion of both the unbound drug and the membrane-bound drug through the plasma membrane (Fig. 5). The authors cannot readily see any possible experimental means by which Model 5 can be distinguished from Model 4: however, it is presented for its academic interest. The transport of drug into the cell interior can be expressed by:

$$\frac{dC_i}{dt} = \frac{3P}{a}\left(C_0 - \frac{C_i}{K}\right) + \frac{3P_m}{a}\left(C_s - \frac{C_i}{K_m}\right) \quad \text{(Eq. 42)}$$

When employing techniques similar to those in the derivation of Model 4, the change in the cell concentration of drug with time is:

$$\frac{dC_c}{dt} = \frac{3P}{a(1 + K_{bm}E)}(F - GC_c) + \frac{3P_m}{a(1 + K_{bm}E)}(M - NC_c) \quad \text{(Eq. 43)}$$

where:

$$M = K_{bm}\left(1 + \frac{1}{K_m}\right)\frac{T}{V_0} \quad \text{(Eq. 44)}$$

$$N = K_{bm}\left(1 + \frac{1}{K_m}\right)\frac{nV_i}{V_0} + \frac{1}{K_m} \quad \text{(Eq. 45)}$$

and P and P_m are the permeability coefficients of the unbound and membrane-bound solute, respectively; K_m is the partition coefficient of the membrane-bound solute; and the terms F, G, E, and others have been previously defined. The solution is:

$$\ln\frac{(PF + P_mM) - (PG + P_mN)C_c}{(PF + P_mM) - (PG + P_mN)C_{c,0}} = -\frac{3(PG + P_mN)}{a(1 + K_{bm}E)}(t) \quad \text{(Eq. 46)}$$

The partition coefficients can be evaluated from the experimental data by the similar procedure in Model 4 and are:

$$K = C_{i,\infty}/C_{0,\infty} = K_{ex} - K_{bm} \quad \text{(Eq. 47)}$$

$$K_m = C_{i,\infty}/C_{s,\infty} = (K_{ex}/K_{bm}) - 1 \quad \text{(Eq. 48)}$$

Model 6—The next two models are useful for evaluating the simultaneous uptake of two radiolabeled solutes. Model 6 assumes that both solutes diffuse independently and that each follows the conditions of Model 1.

Then, the rate of uptake of solutes A and B by a cell can be described by:

$$\frac{dC_{i,A}}{dt} = \frac{3P_A}{a}\left(C_{0,A} - \frac{C_{i,A}}{K_A}\right) \quad \text{(Eq. 49)}$$

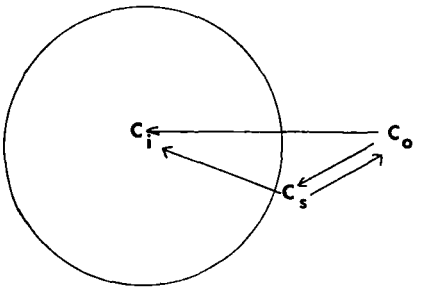

Figure 5—*Model 5: Passive transport of both the unbound solute and the external cell membrane-bound solute across the plasma membrane, with rapid distribution in the heterogeneous cell interior. The cell membrane-bound solute is also in equilibrium with the free solute concentration in the external media.*

and:

$$\frac{dC_{i,B}}{dt} = \frac{3P_B}{a}\left(C_{0,B} - \frac{C_{i,B}}{K_B}\right) \quad \text{(Eq. 50)}$$

where the definitions of the terms are the same as before and the subscripts A and B refer to the two solutes. With Eq. 2:

$$\frac{dC_{i,A}}{dt} = -\frac{3P_A}{a}\left(\frac{1}{K_A} + \frac{nV_i}{V_0}\right)C_{i,A} + \frac{3P_AT_A}{aV_0} \quad \text{(Eq. 51)}$$

and:

$$\frac{dC_{i,B}}{dt} = -\frac{3P_B}{a}\left(\frac{1}{K_B} + \frac{nV_i}{V_0}\right)C_{i,B} + \frac{3P_BT_B}{aV_0} \quad \text{(Eq. 52)}$$

and their second derivatives are:

$$\frac{d^2C_{i,A}}{dt^2} + k_A\frac{dC_{i,A}}{dt} = 0 \quad \text{(Eq. 53)}$$

$$\frac{d^2C_{i,B}}{dt^2} + k_B\frac{dC_{i,B}}{dt} = 0 \quad \text{(Eq. 54)}$$

where:

$$k_A = \frac{3P_A}{a}\left(\frac{1}{K_A} + \frac{nV_i}{V_0}\right) \quad \text{(Eq. 55)}$$

$$k_B = \frac{3P_B}{a}\left(\frac{1}{K_B} + \frac{nV_i}{V_0}\right) \quad \text{(Eq. 56)}$$

The integration of Eq. 53 and the application of the boundary conditions of $C_{i,A} = 0$ at $t = 0$ and $C_{i,A} = C_{i,A,eq}$ at $t = \infty$ yield:

$$C_{i,A} = C_{i,A,eq}(1 - e^{-k_At}) \quad \text{(Eq. 57)}$$

In a similar manner:

$$C_{i,B} = C_{i,B,eq}(1 - e^{-k_Bt}) \quad \text{(Eq. 58)}$$

where $C_{i,A,eq}$ and $C_{i,B,eq}$ are the equilibrium drug concentrations in the cell. By adding Eqs. 57 and 58:

$$C_{i,(A+B)} = C_{i,(A+B)eq} - C_{i,A,eq}e^{-k_At} - C_{i,B,eq}e^{-k_Bt} \quad \text{(Eq. 59)}$$

Experimentally, the concentration of the two radiolabeled solutes in the cell, $C_{i,(A+B)}$, as a function of time and their equilibrium concentration, $C_{i,(A+B)eq}$, are directly measurable. By utilizing the partition coefficients of solutes A and B from independent experiments and knowing the total amounts of each solute added, the equilibrium concentrations of each solute in the cells, $C_{i,A,eq}$ and $C_{i,B,eq}$, can then be calculated, *e.g.*:

$$C_{i,A,eq} = \frac{T_A}{nV_i + (V_0/K_A)} \quad \text{(Eq. 60)}$$

Finally, the respective permeability coefficients can be determined from Eq. 59.

Model 7—This model is similar to Model 6, except that solute *A* follows Model 4 and solute *B* follows Model 1. Accordingly, the solution for Model 7 is:

$$C_{c,(A+B)} = C_{c,(A+B)eq} - C_{c,B,eq}e^{-k_B t} + (C_{s,0} - C_{c,A,eq})e^{-k_A' t} \quad \text{(Eq. 61)}$$

where:

$$k_A' = \frac{3P_A G}{a(1 + K_{bm}E)} \quad \text{(Eq. 62)}$$

Model 8—This model analyzes the situation where the rate-determining barrier to equilibrium drug distribution in the monodispersed cell suspension not only includes the plasma membrane but also the membranes of the cytoplasmic bodies within the cell. The drug distributes instantaneously in the aqueous interior but permeates slowly into the cytoplasmic bodies, including the nucleus.

From Eqs. 2 and 3:

$$\frac{dC_i}{dt} = \frac{3P_e}{a}\left[\frac{T}{V_0} - \left(\frac{nV_i}{V_0} + \frac{1}{K_e}\right)C_i\right] \quad \text{(Eq. 63)}$$

The differentiation of Eq. 63 gives:

$$\frac{d^2C_i}{dt^2} = -\frac{3P_e}{a}\left(\frac{nV_i}{V_0} + \frac{1}{K_e}\right)\frac{dC_i}{dt} \quad \text{(Eq. 64)}$$

The distribution of drug within the aqueous cytoplasmic matrix and cytoplasmic bodies of each cell is:

$$C_i = (1 - \alpha_i)(C_{i,aq} + C^*_{i,aq}) + \alpha_i C_{i,cyt} \quad \text{(Eq. 65)}$$

where α_i is the fraction of cytoplasmic bodies, $(1 - \alpha_i)$ is the fraction of the cytoplasmic aqueous matrix, $C_{i,aq}$ is the unbound drug concentration in the aqueous cell interior, and $C_{i,cyt}$ is the drug concentration in the various cytoplasmic bodies. The concentration adsorbed, $C^*_{i,aq}$, in the aqueous interior is assumed to be linear with respect to the Langmuir isotherm; thus:

$$C^*_{i,aq} = K_{bi}C_{i,aq} \quad \text{(Eq. 66)}$$

where K_{bi} is the linear adsorption constant. Together with Eqs. 65 and 66, it follows that:

$$\frac{dC_i}{dt} = (1 - \alpha_i)(1 + K_{bi})\frac{dC_{i,aq}}{dt} + \alpha_i\frac{dC_{i,cyt}}{dt} \quad \text{(Eq. 67)}$$

The expression for the rate of transfer of drug from the aqueous interior of each cell into the cytoplasmic bodies is given by:

$$\left[\frac{V_0}{K'} + (1 - \alpha_i)V_i\right]\frac{dC_{i,aq}}{dt} = n_i S_i P_{e,cyt}\left(C_{i,aq} - \frac{C_{i,cyt}}{K_{e,cyt}}\right) \quad \text{(Eq. 68)}$$

and:

$$-(1 - \alpha_i)V_i\frac{dC_{i,aq}}{dt} = \alpha_i V_i\frac{dC_{i,cyt}}{dt} \quad \text{(Eq. 69)}$$

where n_i is the total number of cytoplasmic bodies with an average surface area, S_i, possessing an average effective permeability coefficient, $P_{e,cyt}$, and partition coefficient, $K_{e,cyt}$. The partition coefficient K' is the ratio of $C_{i,aq}$ to C_0. A rigorous treatment of Eqs. 68 and 69 should reflect the transport of the drug from the aqueous cytoplasmic matrix into the cytoplasmic bodies, summing over each kind of cytoplasmic bodies with each kind possessing a characteristic permeability and partition coefficient. However, since it is extremely difficult to determine experimentally the particular physical parameters within the cell with any degree of certainty, the average effect is taken as expressed here.

Differentiating Eq. 68 and then using Eq. 69 lead to the following result:

$$\frac{d^2C_{i,aq}}{dt^2} + \beta\frac{dC_{i,aq}}{dt} = 0 \quad \text{(Eq. 70)}$$

where:

$$\beta = \frac{n_i S_i P_{e,cyt}}{\left[\frac{V_0}{K'} + (1 - \alpha_i)V_i\right]}\left[1 + \frac{(1 - \alpha_i)}{\alpha_i K_{e,cyt}}\right] \quad \text{(Eq. 71)}$$

Introducing the boundary conditions of $C_{i,aq} = 0$ and $C_{i,aq(\infty)} = C_{i,aqE}$, where $C_{i,aqE}$ is the equilibrium drug concentration in the aqueous interior of the cell, the solution is:

$$C_{i,aq} = C_{i,aqE}(1 - e^{-\beta t}) \quad \text{(Eq. 72)}$$

and with Eq. 69, the derivative becomes:

$$\frac{dC_{i,aq}}{dt} = -\frac{\alpha_i}{(1 - \alpha_i)}\frac{dC_{i,cyt}}{dt} = C_{i,aqE}\beta e^{-\beta t} \quad \text{(Eq. 73)}$$

Finally, by substituting Eqs. 73 and 67 into Eq. 64, the following second-order, nonhomogeneous differential equation is obtained:

$$\frac{d^2C_i}{dt^2} + \frac{3P_e nV_i}{aV_0}\frac{dC_i}{dt} = -\frac{3P_e}{aK_e}(1 - \alpha_i)K_{bi}C_{i,aqE}\beta e^{-\beta t} \quad \text{(Eq. 74)}$$

Integration from initial to equilibrium conditions yields:

$$C_i = C_{i,E} - \left[\frac{G}{(\alpha - \beta)} + C_{i,eq}\right]e^{-\alpha t} + \frac{G}{(\alpha - \beta)}e^{-\beta t} \quad \text{(Eq. 75)}$$

where:

$$\alpha = \frac{3P_e nV_i}{aV_0} \quad \text{(Eq. 76)}$$

$$G = \frac{3P_e(1 - \alpha_i)K_{bi}C_{i,aqE}}{aK_e(\alpha - \beta)} \quad \text{(Eq. 77)}$$

CONCLUSION

Some physical models are derived for the diffusional transport of drugs across membranes of mammalian cells in culture suspension. These theoretical models represent but a few of the many ways molecules can penetrate cells. To assign mechanisms, models such as these must be used to evaluate the data and to design experiments. The experimental sequel of this theoretical presentation will demonstrate the usefulness of the physical model approach, as will be seen in the transport of various sterols and cardiac glycosides into and out of Burkitt lymphoma cells in culture suspension.

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