DISCUSSION

The data in Fig. 1 show that, for a constant instilled concentration, aqueous humor levels decrease as the instilled volume is decreased. However, aqueous humor drug levels do not decrease in proportion to dose size. For example, decreasing the instilled volume from 25 to 5 μl, a fivefold dosage reduction, results in only about a twofold reduction in the area under the curve of aqueous humor concentration *versus* time. Previous attempts at quantitation of pilocarpine uptake and distribution could not have predicted such an occurrence since available data on tear and instilled solution drainage were not quantitatively considered (1). The model developed here takes this factor into account and is, therefore, able to make reasonable predictions even when instilled volume and/or concentration are altered.

As has been mentioned, the data from Fig. 1 imply that a considerable decrease in instilled volume coupled with a slight increase in instilled concentration should result in the same amount of drug reaching the eye interior as if much larger volumes were administered. The data of Fig. 2 confirm this result, and Fig. 7 shows that the model also predicts it. It is confirmed that the model is sensitive to changes in both instilled volume and/or concentration. Such predictive capability has not previously been available.

Although the mathematics presented here are a simplification of the actual situation, the framework is now available that will allow refinement and addition of more sophisticated experimental data as they become available. Specifically, conjunctival absorption, protein binding, tissue distribution, and more mechanistic data on corneal absorption are needed. As such information becomes available, the treatment presented here can be refined to quantitate it. Furthermore, once all relevant parameters have been identified, the model should lend itself to quantitation of other ophthalmic drugs in addition to pilocarpine.

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Kinetics of Drug Transport and Concurrent Metabolism in Human Cell Cultures I: Theory

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Abstract □ A method for evaluating the passive permeability and single-step metabolism of drugs in suspension cultures of mammalian cells was formulated assuming linear kinetics. It was assumed that the metabolizing enzymes are driven by endogenous substrates present in steady-state quantities. The presence of the drug in radiolabeled tracer quantities was assumed to cause only a small perturbation from the endogenous steady-state operating point. The time course solutions for the drug and its metabolite are given in terms of macroscopic constants, and their physical interpretations are given in terms of metabolic and transport parameters.

Keyphrases □ Drug transport kinetics—evaluated in suspension cultures of mammalian cells, equations derived □ Metabolism, single step—evaluated for drugs in suspension cultures of mammalian cells, equations derived □ Kinetics—drug transport and concurrent metabolism in suspension cultures of mammalian cells, equations derived

The study of drug kinetics at the cellular level represents a microcosm of the traditional field of pharmacokinetics. Such a study, which includes cellular absorption, elimination, and metabolism *in vitro*, might be regarded as academic and possibly not as relevant as clinical studies *in vivo*. However, the ultimate resolution of the cancer problem and certain persistent viral infections may depend on knowledge of chemotherapy and kinetics at the cellular level. If the tumor problem is epigenetic and potentially reversible, as some investigators believe (1), then the ab-

errant neoplastic state would not represent a mutation of genetic material but rather a persistent alteration in genetic expression that might be amenable to control by a new class of chemotherapeutic agents acting at the cellular level.

BACKGROUND

Although current understanding of the molecular biology of the tumor cell is insufficient to define the nature of the "persistent switches" that determine dedifferentiation and tumorigenesis, the interactions of the virus with the human host cell are understood to such a degree that modern antiviral therapy has "come of age" (2). It was only 17 years ago that many investigators believed that the viral and cellular processes were so similar that it would not be possible to design agents to distinguish between them. For these reasons, transport and metabolism at the cellular level must be evaluated.

The thymidine incorporation rate into DNA does not reflect accurately the DNA synthesis rate (3–5). One possible reason for this discrepancy is that the uptake rate of the precursor is rate limiting for its incorporation into the macromolecule in question (3, 6, 7). The simultaneous transport of precursors and the biosynthesis of macromolecules in isolated chloroplasts (8) and *Escherichia coli* B (9) were examined in suspension systems. No attempt was made to determine the limiting step in the incorporation of the precursors into the macromolecule.

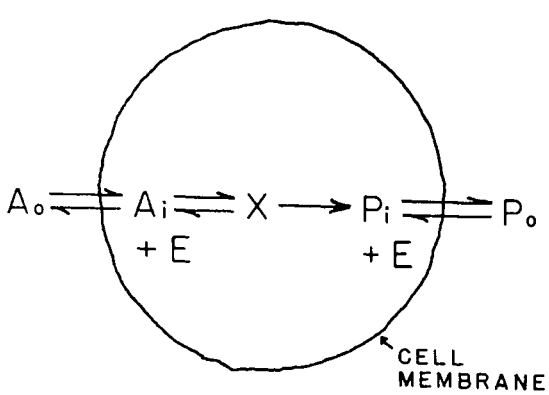
Rashevsky (10) derived an expression for the total diffusional resistance of a spherical cell using an implicit assumption that the partition coefficient between the cell interior and the external medium is unity (11). Hearon (12) noted that Rashevsky's treatment assumed that the metabolism rate is the same at every point in the cell and that the average metabolism is different from zero. Since the cases considered by Rashevsky (10) and Hearon (13) were the zero- and first-order situations for the metabolism rate, Q, Best (11) stated that the actual Michaelis–Menten kinetics will be bound by these two cases.

Blum and Jenden (14) considered this same problem. To obtain the concentration profiles in an intermediate range, they expanded the metabolism rate factor, Q, by a first-order Taylor series and found an expression for the volume-averaged concentration inside of the cell. In their derivation, the value of C^*, the value of the substrate about which the expansion was taken, was arbitrary and was chosen to suit a particular situation.

Several expressions were derived to evaluate diffusional and biosynthetic parameters from experimental data. Best (15) derived an equation for a spherically symmetric metabolizing cell which accounts for transport and metabolism under the "quasisteady-state" assumption (when the metabolism rate and the transport rate are equal). The partition coefficient between the cell and the external medium was assumed to be unity, as in the treatment of Rashevsky (10). Best (16), using baker's yeast, evaluated the parameters for sucrose–invertase and glucose–hexokinase systems at different temperatures with nonlinear regression analysis. In the following discussion, the biosynthetic parameters and the transport parameters will be considered from temporal data.

THEORETICAL

Biosynthetic–Transport Model—Consider the one-step conversion of a drug, A, into a product, P, inside the spherically symmetric cell in a suspension culture. Scheme I depicts the model subsystem of an endogenous biosynthetic pathway that will be considered in this study. In this case, P_i is the terminal product of a biosynthetic pathway that may be sensitive to feedback inhibition. If A_o and P_o are the total amounts of drug and product, respectively, in the fluid compartment, if A_i and P_i are the total amounts of drug and product, respectively, inside the cell, and if X is the total amount of intermediate complex formed by enzyme E with drug A, then Scheme I may be represented by the reaction chain shown in Scheme II.



Scheme I

$$A_o \underset{k_2}{\overset{k_1}{\rightleftharpoons}} A_i + E \underset{k_4}{\overset{k_3}{\rightleftharpoons}} X \xrightarrow{k_5} E + P_i \underset{k_7}{\overset{k_6}{\rightleftharpoons}} P_o$$

Scheme II

This five-compartment system contains two physical and three chemical compartments. The physical compartments are due to the external fluid, denoted by the subscript o, and the cell interior, denoted by the subscript i, of the spherically symmetric cell in suspension; the chemical compartments are due to the three distinct chemical species A, X, and P. In this model, the formation of P_i is assumed to be irreversible, and the enzyme reactions are homogeneous in the cell.

The basic differential equations for this system are:

$$dA_o/dt = -k_1A_o + k_2A_i \quad \text{(Eq. 1)}$$

$$dA_i/dt = -k_2A_i - k_3EA_i + k_1A_o + k_4X \quad \text{(Eq. 2)}$$

$$dX/dt = -(k_4 + k_5)X + k_3EA_i \quad \text{(Eq. 3)}$$

$$dP_i/dt = -k_6P_i + k_5X + k_7P_o \quad \text{(Eq. 4)}$$

$$dP_o/dt = -k_7P_o + k_6P_i \quad \text{(Eq. 5)}$$

The transport parameters k_1, k_2, k_6, and k_7 are derivable from physical considerations, and they can be determined from independent experiments where biosynthesis is not a factor. This fact is important because once k_1, k_2, k_6, and k_7 are determined, the only rate constant that remains to be evaluated from the simultaneous biosynthetic–transport situation involves the biosynthetic process inside the cell. If it is assumed that the rate of change of X is much slower than that of A_i or P_i, Eq. 3 can be written after allowing for a brief, but negligible, induction period:

$$0 = -(k_4 + k_5)X + k_3EA_i \quad \text{(Eq. 6)}$$

or:

$$X = [k_3/(k_4 + k_5)]EA_i \quad \text{(Eq. 7)}$$

If K_m is defined as:

$$K_m = (k_4 + k_5)/k_3 \quad \text{(Eq. 8)}$$

then Eq. 7 becomes:

$$X = K_m^{-1}EA_i \quad \text{(Eq. 9)}$$

Substituting Eq. 9 into Eq. 2 yields:

$$dA_i/dt = -k_2A_i + k_1A_o - k_3EA_i + k_4K_m^{-1}EA_i \quad \text{(Eq. 10)}$$

or:

$$dA_i/dt = -k_2A_i + k_1A_o + (k_4K_m^{-1} - k_3)EA_i \quad \text{(Eq. 11)}$$

However, since $E = E_o - X$, then:

$$E = E_o - K_m^{-1}EA_i \quad \text{(Eq. 12)}$$

where E_o is the total amount of enzyme in the cell, and:

$$E = E_o/(1 + A_i/K_m) \quad \text{(Eq. 13)}$$

Using this expression for E in Eq. 11 yields:

$$dA_i/dt = -k_2A_i + k_1A_o + \frac{(k_4K_m^{-1} - k_3)}{(1 + K_m^{-1}A_i)}E_oA_i \quad \text{(Eq. 14)}$$

Since:

$$k_4K_m^{-1} - k_3 = k_3k_4/(k_4 + k_5) - k_3 = -k_5K_m^{-1} \quad \text{(Eq. 15)}$$

and:

$$V_m \equiv k_5E_o \quad \text{(Eq. 16)}$$

by definition, Eq. 14 becomes:

$$dA_i/dt = k_1A_o - k_2A_i - V_mA_i/(K_m + A_i) \quad \text{(Eq. 17)}$$

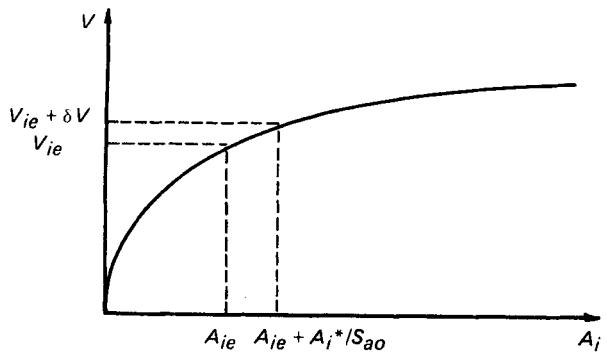


Figure 1—*Perturbation of an enzyme system from the operating point* (A_{ie}, V_{ie}).

Similar considerations applied to Eq. 4 give:

$$dP_i/dt = -k_6P_i + k_7P_o + V_mA_i/(K_m + A_i) \quad \text{(Eq. 18)}$$

Equations 1, 5, 17, and 18 completely describe the reaction chain of Scheme II when the steady state for the enzyme–substrate complex, X, is assumed.

Model Modified for Tracer Analysis—If it is assumed that the enzyme that metabolizes drug A is normally being driven by an endogenous substrate, A_{ie}, then the drug will add a substrate load on this enzyme which might be thought of as a perturbation on A_{ie}.

If A is the total amount of drug in the cell suspension system (the system considered here consists of the fluid and cells contained in an aliquot of volume V) and if A_i and A_o are the total amounts of drug inside of the cell and in the fluid milieu of the cell, then:

$$A = A_i + A_o \quad \text{(Eq. 19)}$$

Similarly, for the total amount of product in the system:

$$P = P_i + P_o \quad \text{(Eq. 20)}$$

Throughout this section, all definitions for A apply equally for P. The major chemical species are usually denoted with a capital letter, and the subscripting of this component is denoted with small letters. The one exception is the definition of specific activity. In this case, the chemical species is denoted with a small letter.

In the external medium, a radiolabeled drug has a specific activity:

$$S_{ao} = A_o^*/A_o \quad \text{(Eq. 21)}$$

where A_o^* is the total amount of labeled drug in the fluid compartment of the system. If it is assumed that the flux of cold drug and the flux of labeled drug are mutually independent, then as the tracer A_o^* enters the cell and becomes A_i^*, a total amount of drug A_i^*/S_{ao} has entered the cell from the external milieu of the cell at a time t. Thus:

$$A_i = A_{ie} + A_i^*/S_{ao} \quad \text{(Eq. 22)}$$

where A_{ie} is the total amount of cold endogenous drug inside the cell under stationary conditions.

Before the labeled drug entered the cell, the enzyme that catalyzed the reaction of A_i into P_i was being driven at a rate V_{ie} by A_{ie}. The increase in A_i by an amount A_i^*/S_{ao} caused the system to be perturbed from A_{ie}. This situation is shown in Fig. 1. Writing the perturbed rate as $V_{ie} + \delta V$ then gives:

$$V_{ie} + \delta V = \frac{V_mA_i}{K_m + A_i} \quad \text{(Eq. 23)}$$

$$V_{ie} + \delta V = \frac{V_m(A_{ie} + A_i^*/S_{ao})}{K_m + (A_{ie} + A_i^*/S_{ao})} \quad \text{(Eq. 24)}$$

$$V_{ie} + \delta V = \frac{V_mA_{ie}}{K_m + (A_{ie} + A_i^*/S_{ao})} + \frac{V_mA_i^*/S_{ao}}{K_m + (A_{ie} + A_i^*/S_{ao})} \quad \text{(Eq. 25)}$$

If it is assumed that the endogenous drug level is much larger than the amount A_i^*/S_{ao}, *i.e.*, $A_{ie} \gg A_i^*/S_{ao}$, then the equation becomes:

$$V_{ie} + \delta V \doteq V_mA_{ie}/(K_m + A_{ie}) + KA_i^*/S_{ao} \quad \text{(Eq. 26)}$$

where:

$$K \equiv V_m/(K_m + A_{ie}) \quad \text{(Eq. 27)}$$

But:

$$V_{ie} = V_mA_{ie}/(K_m + A_{ie}) \quad \text{(Eq. 28)}$$

Therefore:

$$\delta V = KA_i^*/S_{ao} \quad \text{(Eq. 29)}$$

Applying this result to Eq. 17 gives:

$$d(A_{ie} + A_i^*/S_{ao})/dt = k_1(A_{ox} + A_o^*/S_{ao}) - k_2(A_{ie} + A_i^*/S_{ao}) - V_{ie} - \delta V \quad \text{(Eq. 30)}$$

where A_{ox} is the total amount of cold precursor in the fluid milieu of the cells in the absence of A_o^*/S_{ao} under stationary conditions. Since:

$$dA_{ie}/dt = k_1A_{ox} - k_2A_{ie} - V_{ie} \quad \text{(Eq. 31)}$$

then:

$$dA_i^*/dt = k_1A_o^* - k_2A_i^* - S_{ao}\,\delta V \quad \text{(Eq. 32)}$$

and using Eq. 29 to eliminate δV yields:

$$dA_i^*/dt = k_1A_o^* - (k_2 + K)A_i^* \quad \text{(Eq. 33)}$$

Similarly, Eqs. 1, 18, and 5 become:

$$dA_o^*/dt = -k_1A_o^* + k_2A_i^* \quad \text{(Eq. 34)}$$

$$dP_i^*/dt = -k_6P_i^* + k_7P_o^* + KA_i^* \quad \text{(Eq. 35)}$$

$$dP_o^*/dt = -k_7P_o^* + k_6P_i^* \quad \text{(Eq. 36)}$$

respectively.

Closed System Requirement—For a closed system, it is necessary that:

$$T^* = A^* + P^* = \text{constant} \quad \text{(Eq. 37)}$$

where T^* is the total amount of label in the system and:

$$A^* = A_o^* + A_i^* \quad \text{(Eq. 38)}$$

$$P^* = P_o^* + P_i^* \quad \text{(Eq. 39)}$$

Adding Eqs. 33 and 34 yields:

$$dA^*/dt = -KA_i^* \quad \text{(Eq. 40)}$$

and adding Eqs. 35 and 36 gives:

$$dP^*/dt = KA_i^* \quad \text{(Eq. 41)}$$

But since:

$$dT^*/dt = dA^*/dt + dP^*/dt = 0 \quad \text{(Eq. 42)}$$

it can be seen that Eqs. 40 and 41 are redundant and really express the same condition. For a closed system, therefore, the following set of linear differential equations must be solved:

$$dA_i/dt = k_1A_o - (k_2 + K)A_i \quad \text{(Eq. 43)}$$

$$dP_i/dt = -k_6P_i + k_7P_o + KA_i \quad \text{(Eq. 44)}$$

$$dA/dt = -KA_i \quad \text{(Eq. 45)}$$

which are subject to the constraint that:

$$T = A + P \quad \text{(Eq. 46)}$$

In Eqs. 43–46, the label * has been omitted since all time-dependent variables refer to labeled quantities. In the remainder of this section and in those to follow, this will be understood. Since:

$$P = P_o + P_i \quad \text{(Eq. 47)}$$

$$A = A_o + A_i \quad \text{(Eq. 48)}$$

and with the constraint of Eq. 46 in Eqs. 47 and 48, it is found that:

$$P_o = T - A - P_i \quad \text{(Eq. 49)}$$

$$A_o = A - A_i \quad \text{(Eq. 50)}$$

Substituting Eqs. 49 and 50 into Eqs. 44 and 43, respectively, yields:

$$dA_i/dt = k_1A - (k_1 + k_2 + K)A_i \quad \text{(Eq. 51)}$$

$$dP_i/dt = k_7T - k_7A + KA_i - (k_6 + k_7)P_i \quad \text{(Eq. 52)}$$

Equations 45, 51, and 52 form a system of linear differential equations in three variables, A, A_i, and P_i.

In the next section, the solutions to this system of equations will be examined. One final remark, however, is in order with regard to the apparent enzyme rate constant K. Equation 27 can be rewritten as:

$$1/K = K_m/V_m + A_{ie}/V_m \qquad \text{(Eq. 53}a\text{)}$$

so that a plot of $1/K$ *versus* A_{ie} is linear; from such a plot, V_m and K_m can be determined. Since $V_m = k_5E_o$, Eq. 27 can be rewritten as:

$$K = \frac{k_5}{K_m + A_{ie}} E_o \qquad \text{(Eq. 53}b\text{)}$$

so k_5 can be determined from a linear plot of K *versus* E_o if A_{ie} is kept constant and K_m and A_{ie} are known. It remains to be seen if A_{ie} and E_o can be changed experimentally. If these parameters can be varied, then it is apparently possible to determine the fundamental rate parameters of the enzyme catalysis within the intact cell.

Solutions to Closed System Equations—The following assumptions were made to derive the differential equations, Eqs. 45, 51, and 52, for the closed system.

First, it was assumed that the rate of change of the enzyme–substrate complex, X, is much slower than the rate of change of either A_i or P_i within the cell; *i.e.*, transport of the substrate is much faster than the bioconversion of the substrate. This assumption was utilized to obtain an algebraic constraint on X (Eq. 9). In this model, the biosynthetic reaction is taken to be homogeneous within the cell.

Next, it was assumed that the endogenous levels of substrate within the cells, A_{ie}, did not change during the experiment. Thus, the cells must reach a steady state with respect to the synthesis of the endogenous product. In the light of feedback inhibition and other intracellular regulatory mechanisms, this assumption seems reasonable when the cells are not subject to sudden stress.

By using this constancy of A_{ie} as a steady-state operating point and assuming that the total amount of substrate that diffuses into the cells from the fluid media, A_i/S_{ao}, is much less than A_{ie}, the enzymatic rate of change of the substrate reduces to the linear expression (Eq. 26) with respect to its A_i* dependence. The effective linear rate constant for this enzyme reaction is given in Eq. 53*b*. In this derivation, it is implicit that only one substrate is involved that can limit the rate of the enzyme reaction. However, many biological reactions need a second and, sometimes, a third substrate.

Finally, it was assumed that the substrate and the product form a closed system such that the conversion of the ^{14}C-label can be assumed in the form described by Eqs. 37–39. If there are other routes of loss of the ^{14}C-label, then this conservation relationship cannot hold.

The initial and the final assumptions provided algebraic constraints that reduce the original five-compartment system to the three-compartment system expressed by Eqs. 45, 51, and 52. The solutions to this system of equations are derived in the *Appendix* and are:

$$A_i = A_{ib}e^{-\beta t} + A_{ia}e^{-\alpha t} \qquad \text{(Eq. 54)}$$

$$A = A_be^{-\beta t} + A_ae^{-\alpha t} \qquad \text{(Eq. 55)}$$

$$P_i = P_i(\infty) - P_{ib}e^{-\beta t} - P_{id}e^{-\delta t} - P_{ia}e^{-\alpha t} \qquad \text{(Eq. 56)}$$

where:

$$\alpha = \gamma/2 + \sqrt{(\gamma/2)^2 - Kk_1} \qquad \text{(Eq. 57)}$$

$$\beta = \gamma/2 - \sqrt{(\gamma/2)^2 - Kk_1} \qquad \text{(Eq. 58)}$$

$$\gamma = \alpha + \beta = k_1 + k_2 + K \qquad \text{(Eq. 59)}$$

$$\delta = k_6 + k_7 \qquad \text{(Eq. 60)}$$

$$A_{ib} = \frac{k_1A(0) - \beta A_i(0)}{\alpha - \beta} \qquad \text{(Eq. 61)}$$

$$A_{ia} = \frac{\alpha A_i(0) - k_1A(0)}{\alpha - \beta} \qquad \text{(Eq. 62)}$$

$$A_b = \frac{\alpha A(0) - KA_i(0)}{\alpha - \beta} \qquad \text{(Eq. 63)}$$

$$A_a = \frac{KA_i(0) - \beta A(0)}{\alpha - \beta} \qquad \text{(Eq. 64)}$$

$$P_b = \left(\frac{k_7 - \beta}{\delta - \beta}\right) A_b \qquad \text{(Eq. 65)}$$

$$P_a = \left(\frac{k_7 - \beta}{\alpha - \delta}\right) A_a \qquad \text{(Eq. 66)}$$

$$P_d = \frac{1}{(\delta - \beta)(\alpha - \delta)}\left\{1 - \frac{k_7}{k_6 + k_7}\alpha\beta T + (\gamma - \delta)(k_7 + \delta)P_i(0) - k_6KA_i(0)\right\} \qquad \text{(Eq. 67)}$$

$$P_i(\infty) = \frac{k_7T}{k_6 + k_7} \qquad \text{(Eq. 68)}$$

In these expressions, $A(0)$ and $A_i(0)$ are the values of A and A_i at time 0; $P_i(\infty)$ is the value of P_i at time ∞. The expression for A_o can be derived from Eqs. 38 and 54 and is given by:

$$A_o = (A_b - A_{ib})e^{-\beta t} + (A_a - A_{ia})e^{-\alpha t} \qquad \text{(Eq. 69)}$$

Similarly, P_o can be derived from Eqs. 37–39 and 54–56. This expression is not needed here.

In the described formulation, all quantities were computed in terms of the amount of the substance in a particular compartment for a sample of suspension, containing cells and fluid, of volume V. Volume V can be expressed as:

$$V = V_o + nV_i \qquad \text{(Eq. 70)}$$

where n is the number of cells in V, V_i is the volume of one cell, and V_o is the fluid volume. These equations can be converted to concentration units by dividing by the product of the volumes involved. For example, Eq. 61 would become, in concentration units:

$$[A_{ib}] = \frac{\left(\frac{V}{nV_i}\right)k_1[A(0)] - \beta[A_i(0)]}{\alpha - \beta} \qquad \text{(Eq. 71)}$$

where [ ] indicate the concentration of the quantity within the mass balance equations, Eqs. 47 and 48, become:

$$P = V_o[P_o] + nV_i[P_i] \qquad \text{(Eq. 72)}$$

$$A = V_o[A_o] + nV_i[A_i] \qquad \text{(Eq. 73)}$$

The solutions for A_i and A_o given by Eqs. 54 and 69, respectively, tend to zero as time tends to infinity. This behavior is expected for the substrate in the system when the reaction has been completed. The solutions for P_i and P_o, on the other hand, must tend to $P_i(\infty)$ and $P_o(\infty)$, respectively, as t tends to infinity. This means that:

$$T = P_i(\infty) + P_o(\infty) \qquad \text{(Eq. 74)}$$

from Eq. 37. Substituting Eq. 74 into Eq. 68 yields:

$$P_i(\infty) = \frac{k_7}{k_6 + k_7}[P_i(\infty) + P_o(\infty)] \qquad \text{(Eq. 75}a\text{)}$$

or:

$$\frac{P_i(\infty)}{P_o(\infty)} = \frac{k_7}{k_6} \qquad \text{(Eq. 75}b\text{)}$$

Expressing Eq. 75*b* in terms of concentration units gives:

$$\frac{[P_i(\infty)]}{[P_o(\infty)]} = \frac{V_ok_7}{nV_ik_6} \qquad \text{(Eq. 76)}$$

Turi (17) defined the intrinsic partition coefficient κ_p of a compound P between the cell fraction of n cells, each of volume V_i, and the fluid media of volume V_o as the ratio of the concentrations expressed in Eq. 76, *i.e.*:

$$\kappa_p = \frac{[P_i(\infty)]}{[P_o(\infty)]} \qquad \text{(Eq. 77)}$$

Since the relationship between the amounts and the concentrations of P_i and P_o at time ∞ are:

$$P_i(\infty) = nV_i[P_i(\infty)] \qquad \text{(Eq. 78)}$$

$$P_o(\infty) = V_o[P_o(\infty)] \qquad \text{(Eq. 79)}$$

and with Eqs. 74 and 77–79, it is found that:

$$P_i(\infty) = \frac{TnV_i\kappa_p}{(nV_i\kappa_p) + V_o} \qquad \text{(Eq. 80)}$$

$$P_o(\infty) = \frac{TV_o}{(nV_i\kappa_p) + V_o} \qquad \text{(Eq. 81)}$$

$$[P_i(\infty)] = \frac{\kappa_pT}{(nV_i\kappa_p) + V_o} \qquad \text{(Eq. 82)}$$

$$[P_o(\infty)] = \frac{T}{(nV_i\kappa_p) + V_o} \qquad \text{(Eq. 83)}$$

Equations 80–83 can be used to determine these asymptotic values of P_i and P_o from data independent of the biosynthetic or transport kinetics.

Determination of Transport Parameters—The transport parameters k_1, k_2, k_6, and k_7 can be derived in terms of physical constants. The expressions for these parameters are:

$$k_1 = \frac{3P_a}{a}\frac{nV_i}{V_o} \quad \text{(Eq. 84)}$$

$$k_2 = \frac{3P_a}{a\kappa_a} \quad \text{(Eq. 85)}$$

$$k_6 = \frac{3P_p}{a\kappa_p} \quad \text{(Eq. 86)}$$

$$k_7 = \frac{3P_p}{a}\frac{nV_i}{V_o} \quad \text{(Eq. 87)}$$

where a is the radius of the cell and P_a, P_p, and κ_p are the intrinsic permeability and partition coefficients of the drug and product, respectively. These parameters are very important because, by suitably arranging the experimental situation, they can be determined independently from the biosynthetic reaction. Thus, in the simultaneous biosynthetic–transport situation, the only parameters that remain to be determined are those associated with the enzymatic process. Turi (17) found that when fetal bovine serum was used in the medium of the uptake and release transport experiments, the binding of many drugs could be accounted for by assuming a linear binding model of the drug to the fetal bovine component of the medium. In this way, in the presence of fetal bovine serum, the effective permeability, P_{ce}, and the effective partition coefficient, κ_{ce}, of a drug C are the parameters that are measured experimentally. These are related to the intrinsic parameters P_c and κ_c by the expressions:

$$P_{ce} = \frac{P_c}{1 + \rho_b[S]} \quad \text{(Eq. 88)}$$

$$\kappa_{ce} = \frac{\kappa_c}{1 + \rho_b[S]} \quad \text{(Eq. 89)}$$

where ρ_b is the equilibrium serum–drug binding constant.

APPENDIX: SOLUTIONS TO BIOCONVERSION–TRANSPORT EQUATIONS

The equations to be solved are 45, 51, and 52. Substituting $\gamma = k_1 + k_2 + K$ in Eq. 51 yields:

$$dA_i/dt = -\gamma A_i + k_1 A \quad \text{(Eq. A1)}$$

Substituting $\delta = k_6 + k_7$ in Eq. 52 yields:

$$dP_i/dt = KA_i - k_7 A - \delta P_i + k_7 T \quad \text{(Eq. A2)}$$

Taking the Laplace transform of Eqs. A1, 45, and A2 yields, respectively:

$$(s + \gamma)a_i - k_1 a = A_i(0) \quad \text{(Eq. A3)}$$

$$Ka_i + sa = A(0) \quad \text{(Eq. A4)}$$

$$-Ka_i + k_7 a + (s + \delta)p_i = k_7 T/s + P_i(0) \quad \text{(Eq. A5)}$$

Since the determinant (det) of the matrix for a_i, a, and p_i is:

$$\det = (s + \beta)(s + \alpha)(s + \delta) \quad \text{(Eq. A6)}$$

where $\alpha = \gamma/2 + (\gamma/2)^2 - Kk_1$, $\beta = \gamma/2 - (\gamma/2)^2 - Kk_1$, $\alpha\beta = Kk_1$, $\gamma - \alpha = \beta$, and $\gamma - \beta = \alpha$. The solutions for a_i, a, and p_i can be found from Cramer's rule and are:

$$a_i = (s + \beta)^{-1}(s + \alpha)^{-1}k_1 A(0) + sA_i(0) \quad \text{(Eq. A7)}$$

$$a = (s + \beta)^{-1}(s + \alpha)^{-1}(s + \gamma)A(0) - KA_i(0) \quad \text{(Eq. A8)}$$

$$p_i = (s + \beta)^{-1}(s + \alpha)^{-1}(s + \delta)^{-1}A_i(0)\Delta_{13} - A(0)\Delta_{23} + P_i(0)\Delta_{33} + (s + \beta)^{-1}(s + \alpha)^{-1}(s + \delta)^{-1}s^{-1}k_7 T\Delta_{33} \quad \text{(Eq. A9)}$$

where the Δ_{ij} are the minors of the ijth element of the matrix formed from a_i, a, and p_i. Taking the inverse transforms yields:

$$A_i = A_{ib}e^{-\beta t} + A_{ia}e^{-\alpha t} \quad \text{(Eq. A10)}$$

$$A = A_b e^{-\beta t} + A_a e^{-\alpha t} \quad \text{(Eq. A11)}$$

$$P_i = P_i(\infty) - P_{ib}e^{-\beta t} - P_{id}e^{-\delta t} - P_{ia}e^{-\alpha t} \quad \text{(Eq. A12)}$$

$$\alpha = \gamma/2 + (\gamma/2)^2 - Kk_1 \quad \text{(Eq. A13)}$$

$$\beta = \gamma/2 - \sqrt{(\gamma/2)^2 - Kk_1} \quad \text{(Eq. A14)}$$

$$\gamma = \alpha + \beta = k_1 + k_2 + K \quad \text{(Eq. A15)}$$

$$\delta = k_6 + k_7 \quad \text{(Eq. A16)}$$

$$A_{ib} = \frac{k_1 A(0) - \beta A_i(0)}{\alpha - \beta} \quad \text{(Eq. A17)}$$

$$A_{ia} = \frac{\alpha A_i(0) - k_1 A(0)}{\alpha - \beta} \quad \text{(Eq. A18)}$$

$$A_b = \frac{\alpha A(0) - KA(0)}{\alpha - \beta} \quad \text{(Eq. A19)}$$

$$A_a = \frac{KA_i(0) - \beta A(0)}{\alpha - \beta} \quad \text{(Eq. A20)}$$

$$P_b = \left(\frac{k_7 - \beta}{\delta - \beta}\right) A_b \quad \text{(Eq. A21)}$$

$$P_a = \left(\frac{k_7 - \beta}{\alpha - \delta}\right) A_a \quad \text{(Eq. A22)}$$

$$B = \frac{1}{(\delta - \beta)(\alpha - \delta)}\left\{\left[1 - \frac{k_7}{k_6 + k_7}\right]\alpha\beta T + (\gamma - \delta)(k_7 + \delta)P_i(0) - k_6 K A_i(0)\right\} \quad \text{(Eq. A23)}$$

$$P_i(\infty) = \frac{k_7 T}{k_6 + k_7} \quad \text{(Eq. A24)}$$

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