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A COMPARISON OF STOCHASTIC AND DETERMINISTIC MODELS FOR CELL MEMBRANE TRANSPORT

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ABSTRACT

This paper describes several models for enzyme mediated transport across a cell membrane. A standard Michaelis-Menten model (based on empirical descriptions) is compared to a deterministic model (based on the idealization of the plasma membrane as a tri-lamellar structure) and to a stochastic model (having the additional assumption of compartmentation of the tri-lamellar membrane). Lineweaver-Burke plots for each model are compared using two different sets of parameter values. These plots suggest, and analytic investigations of the models show, that the deterministic formulations yield different transport kinetics than does the stochastic formulation. The effect of the application of the concept of compartmentation to some stochastic models of *in vivo* systems is briefly discussed.

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1. INTRODUCTION

This paper describes a model for enzyme mediated transport across a cell membrane. A major assumption of the model is that the membrane is divided into independent compartments and that each compartment contains exactly one enzyme molecule. A stochastic model for transport across the membrane is developed and the rate of transport is found. It is shown that deterministic formulations of the model yield different transport kinetics.

Stochastic models for chemical reactions are developed to study the role of random fluctuations in concentrations of chemical reactants in small subvolumes of a reaction volume. A basic assumption in most of these models is that the reaction takes place in a well mixed volume: that is, changes in concentrations in small subvolumes will effect the concentrations in the entire volume. Stochastic models for specific chemical reactions that implicitly include this assumption have been studied by Staff (1970), Heyde and Heyde (1969), Smith (1971), Darvey, Ninham and Staff (1966), Oppenheim, Shuler and Weiss (1969) and others. All these reactions have the following property in common: if N denotes the number of molecules in the system, then in the limit as $N \to \infty$, the corresponding stochastic and deterministic models yield the same results. (It should be pointed out that some special chemical reactions do not have this property (Singer, 1953).) Heyde and Heyde (1971) have shown that for the one-substrate, one-product enzyme system the difference between the stochastic and deterministic models is negligible for most experimental situations. Since the enzyme reaction *in vivo* occurs in a highly structured system, the cell, there is little reason to assume a well mixed reaction volume. An objective of this paper is to indicate by example that if the assumption of a well mixed volume is abandoned, the stochastic and deterministic models can yield significantly different reaction kinetics.

We now outline the basic structure of the membrane model to be investigated. A complete, unequivocal characterization of membrane ultrastructure seems, at present, to be an impossible task (Hendler, 1971; van Bruggen, 1971). However, at least for some membranes, a "unit membrane" structure (Robertson, 1959) with functional, or even structural subunits (Green and Perdue, 1966) seems to be a workable hypothesis. Rothfield and Romeo (1971) discuss the concept of a functional subunit consisting of an individual enzyme molecule together with the phospholipid molecules in its immediate vicinity. Thus we envision a tri-lamellar membrane divided into identical compartments (subunits). Each compartment consists of three major components: the outer boundary, the inner boundary and the middle region. The concentration of substrate, S_0, outside the membrane's outer boundary is assumed to remain constant. Substrate diffusses into and out of the middle region across the outer boundary. A single enzyme molecule is located on the interior boundary of each compartment. Transport across the inner boundary takes place when the substrate reacts with the enzyme molecule in a simple enzyme-catalyzed reaction (the product of this reaction need not be different from the substrate). The structure of the membrane transport reaction in this compartment can best be described by the following diagram.

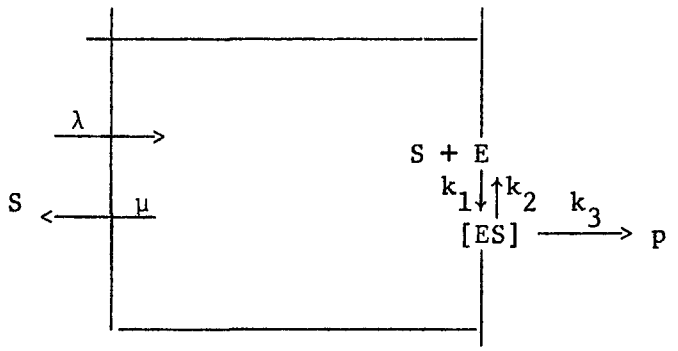


FIGURE 1: Schematic cross-sectional diagram of an idealized plasma membrane showing proposed mechanisms of transport.

Where λ and μ are diffusion rates across the outer boundary and the constants k_1, k_2 and k_3 are the usual first and second order reaction rates associated with the simple Michaelis-Menten model for an enzyme reaction.

Since we assume that all compartments are identical and independent the rate of transport can be investigated by finding the kinetic properties of a single compartment. In Section 2, we develop a stochastic model for a single compartment. In Section 3, we compare this model with two deterministic models for enzyme mediated transport.

2. A STOCHASTIC MODEL FOR THE COMPARTMENT REACTION

In formulating a stochastic or deterministic model for a chemical reaction one implicitly makes additional *ad hoc* assumptions about the physical model. A brief discussion of this problem is given in Oppenheim, Shuler and Weiss (1969). Basically, both deterministic and stochastic models are intuitive approximations to a physical system whose exact properties, including interaction dynamics and diffusion of individual particles within the system, are difficult to analyse. If we assume that each compartment in our membrane model is well mixed in the sense that molecules diffusing across the compartment boundary are rapidly dispersed throughout the compartment, then the stochastic model that we develop below seems intuitively reasonable.

We now develop in detail the stochastic model for a single compartment of the membrane. Let the random variable $N(t)$ denote the number of substrate molecules in the compartment at time t. Let $E(t)$ denote the state at time t of the single enzyme in the compartment: $E(t) = 0$ if the enzyme is not complexed with a substrate molecule and $E(t) = 1$ if the enzyme is complexed with the substrate. We assume that $\{(N(t),E(t)): 0 \leq t\}$ is a continuous

time discrete state Markov process. Under this Markov assumption the single compartment model is completely specified by the infinitesimal transition probabilities: the probability that the transition $(N,E) \to (N',E')$ occurs in the interval $(t, t + \Delta t)$. The model is summarized by the following table of infinitesimal transition probabilities

Reaction	Transition	Probability
$S' \xrightarrow{\lambda} S$	$(N,E) \to (N+1, E)$	$S_0\lambda\Delta t + o(\Delta t)$
$S' \xleftarrow{\mu} S$	$(N,E) \to (N-1, E)$	$\mu N\Delta t + o(\Delta t)$
$E+S \xrightarrow{k_1} ES$	$(N,0) \to (N-1, 1)$	$k_1 N\Delta t + o(\Delta t)$
$ES \xrightarrow{k_2} E+S$	$(N,1) \to (N+1,0)$	$k_2 \Delta t + o(\Delta t)$
$ES \xrightarrow{k_3} E+P$	$(N,1) \to (N, 0)$	$k_3 \Delta t + o(\Delta t)$

The probability that any other transition occurs in the interval $(t, t + \Delta t)$ is $o(\Delta t)$. For notational convenience the second order reaction rate k_1, has concentration units in molecules per compartment volume. Once the compartment volume is determined one can translate the usual reaction rates with concentration units in say moles per liter into the reaction rates used in this paper. S_0 is the constant concentration of substrate in the medium surrounding the exterior boundary of the membrane. S_0 is again in units of molecules per compartment volume. In experimental situations one varies the concentration S_0 and then measures the steady state rate of transport across the membrane. The objective of our analysis will be to compute the rate of transport for a given concentration of substrate in the exterior medium.

Let $p(n,e;t)$ denote the probability that the system is in state (n,e) at time t

$$p(n,e;t) = \Pr\{(N(t),E(t)) = (n,e)\}.$$

From the infinitesimal transition probabilities one can obtain by standard methods the forward Kolmogorov differential equations for $p(n,e;t)$:

$$p'(n,0;t) = -(\lambda^*+\mu n+nk_1)p(n,0;t) + \lambda^* p(n-1,0;t) + \mu(n+1)p(n+1,0;t) + k_2 p(n-1,1;t) + k_3 p(n,1;t) \quad (1)$$

and

$$p'(n,1;t) = -(\lambda^*+\mu n+k_2+k_3)p(n,1;t) + \lambda^* p(n-1,1;t) + \mu(n+1)p(n+1,1;t) + k_1(n+1)p(n+1,0;t). \quad (2)$$

Where $p'(n,e;t)$ is the derivative of $p(n,e;t)$ with respect to t and

$$\lambda^* = \lambda S_0. \quad (3)$$

We define the generating functions

$$F_e(z,t) = \sum_{n=0}^{\infty} z^n p(n,e;t) \quad \text{for} \quad e = 0,1.$$

Multiplying equation (1) by z^n and summing over $n = 0,1,2 \ldots$ we obtain

$$\frac{\partial F_0(z,t)}{\partial t} = \mu(1-z)\frac{\partial F_0(z,t)}{\partial z} + \lambda^*(z-1)F_0(z,t) + k_2 z F_1(z,t) - k_1 z \frac{\partial F_0(z,t)}{\partial z} + k_3 F_1(z,t). \quad (4)$$

Similarly, multiplying equation (2) by z^n and summing over $n = 0,1,2 \ldots$ yields

$$\frac{\partial F_1(z,t)}{\partial t} = \mu(1-z)\frac{\partial F_1(z,t)}{\partial z} + \lambda^*(z-1)F_1(z,t) - (k_2+k_3)F_1(z,t) + k_1 \frac{\partial F_0(z,t)}{\partial z}. \quad (5)$$

Let $p(n,e)$ denote the stationary or limiting distribution of the process $\{(N(t),E(t))\}$,

$$p(n,e) = \lim_{t \to \infty} p(n,e;t)$$

and let

$$F_e(z) = \lim_{t \to \infty} F_e(z,t) \quad \text{for} \quad e = 0,1.$$

The generating functions $F_e(z)$, $e = 0,1$, satisfy equations (4) and (5) with the left hand side set equal to zero. In the appendix, we solve this system of linear first order ordinary differential equations.

Once the generating functions $F_e(z)$, $e = 0,1$, are obtained, the rate of transport across the membrane can be found. The transport of a substrate molecule across the inner boundary corresponds to the transition $(n,1) \to (n,0)$ in the process $\{(N(t),E(t))\}$. The probability that this transition occurs in the time period $(t, t + \Delta t)$ is

$$k_3 \Delta t \Pr\{E(t) = 1\} + o(\Delta t).$$

Thus at equilibrium the rate of transport for a given concentration of substrate, denoted by $V_s(S_0)$, is

$$V_s(S_0) = \lim_{t \to \infty} k_3 \Pr(E(t) = 1) = k_3 F_1(1) \tag{6}$$

where F_1 is given in equation (A.11) of the appendix. Again since the compartments are assumed to be independent and identical the rate of transport for L compartments is just $L \cdot V_s(S_0)$.

From the generating function for the stationary distribution one can obtain other stochastic properties of the system such as the expected value and the variance of the number of substrate molecules in the compartment. These properties depend on complicated expressions involving confluent hypergeometric functions. The results will not be given here since our main interest is the kinetics of the compartment system. In Section 3, we compare our model with two deterministic models for enzyme mediated transport.

3. DETERMINISTIC MODELS AND COMPARISONS

In Section 2, we obtained the transport rate for our stochastic model; we now wish to examine situations in which the kinetics of the stochastic system differ significantly from the usual deterministic results. One could formulate several deterministic models that would be appropriate for comparison with our stochastic model. However, for the purposes of this discussion we will investigate only two deterministic models: a simple Michaelis-Menten model and a deterministic model having the same reaction rates as the stochastic model.

Empirical descriptions of membrane transport sometimes follow standard Michaelis-Menten kinetics (Stein, 1967). Basically, in terms of our model, this implies that the enzyme is exposed to the constant concentration of substrate, S_0, in the surrounding medium. In an intuitive way, this corresponds to the stochastic situation without the compartmental assumption, but with the relation $\lambda = \mu = \infty$.

Let $V_m(S_0)$ denote the Michaelis-Menten transport velocity with the initial enzyme concentration equal to unity,

$$V_m(S_0) = \frac{k_3}{1 + K/S_0} \tag{7}$$

where

$$K = \frac{k_2 + k_3}{k_1} . \tag{8}$$

One can, in a straightforward fashion, form other deterministic rate equations, these equations being based on reactions indicated in Figure 1. Let e,c and s denote the steady-state concentrations of free enzyme, complexed enzyme and substrate respectively. Again for convenience, the units of concentration are in molecules per compartment volume. When the system is

in the steady-state, e, c and s satisfy the following rate equations:

$$\begin{aligned} 0 &= (k_2+k_3)c - k_1 s\circ e \\ 0 &= \lambda S_0 - \mu s - k_1 s\circ e + k_2 c \end{aligned} \tag{9}$$

where S_0 is the constant concentration of substrate outside the outer boundary.

Solving for the deterministic transport rate we obtain

$$V_d(S_0) = \frac{k_3\lambda^*(1-\rho)}{f + \frac{k_3}{2}\left\{1 - \frac{f}{k_3} + \left[\left[1 + \frac{f}{k_3}\right]^2 - \frac{4\lambda^*}{k_3}(1-\rho)^2\right]^{\frac{1}{2}}\right\}} \tag{10}$$

where

$$\rho = \frac{\mu}{\mu+k_1} \quad \text{and} \quad f = \lambda^*(1-\rho) + \rho k_2 \tag{11}$$

and where λ^* is defined in equation (3). This transport rate is compared to the stochastic transport rate, $V_s(S_0)$, when we present our numerical results.

The deterministic model represented by the steady state rate equations, (9), clearly does not correspond exactly to the stochastic model we are investigating. In the deterministic formulation, we have implicitly assumed that the concentrations of the various chemical species within the middle region are differentiable functions of time, whereas in reality they must be step functions. Furthermore, this deterministic formulation ignores the heterogeneous subvolume effect mentioned in Section 1. In fact, this formulation would more accurately reflect a situation in which there were no boundaries between compartments and the enzyme was located on the inner boundary of the cell membrane. The number of enzyme molecules in the reaction volume (middle region of the membrane), composed of many compartment volumes, would then be large. Thus it might then be reasonable to assume the steady state equations, (9), hold.

Lineweaver-Burke plots of $1/V$ versus $1/S_0$ are useful in evaluating and comparing the kinetic behaviors of the three models, despite the statistical difficulties inherent in that particular linearization procedure (Dowd and Riggs, 1965). With this plot, Michaelis-Menten kinetics are linear and the kinetics of the stochastic and deterministic models are non-linear.

In Figure 2, we graph k_3 times the inverse of the transport rate, as a function of $1/S_0$, for all three models. In Figure 2a, k_1, k_2, k_3, μ and λ are equal respectively to 10^4, 10^4, 10^2, 10^2 and 10^2 where the units of concentration are in molecules per compartment volume. In Figure 2b, all rates are the same except that k_1 is 10^5 compartment volumes per molecule per second. If we assume that the volume of a compartment is on the order of 10^{-18} to 10^{-20} cubic centimeters, then the above reaction rates fall within the general range often observed for enzyme reactions in solution. The parameters used in Figure 2 were chosen to demonstrate differences between the stochastic and deterministic formulation, but still fall within the range mentioned above. However, as Laidler and Sundaram (1971) point out, the magnitudes of the rate constants measured from reactions in solution may not be the same as those constants for enzymes bound to membranes *in vivo*.

Figure 2 suggests linearity of $1/V_d$ and $1/V_s$ as $1/S_0 \to \infty$. Indeed, for large $1/S_0$ one can write

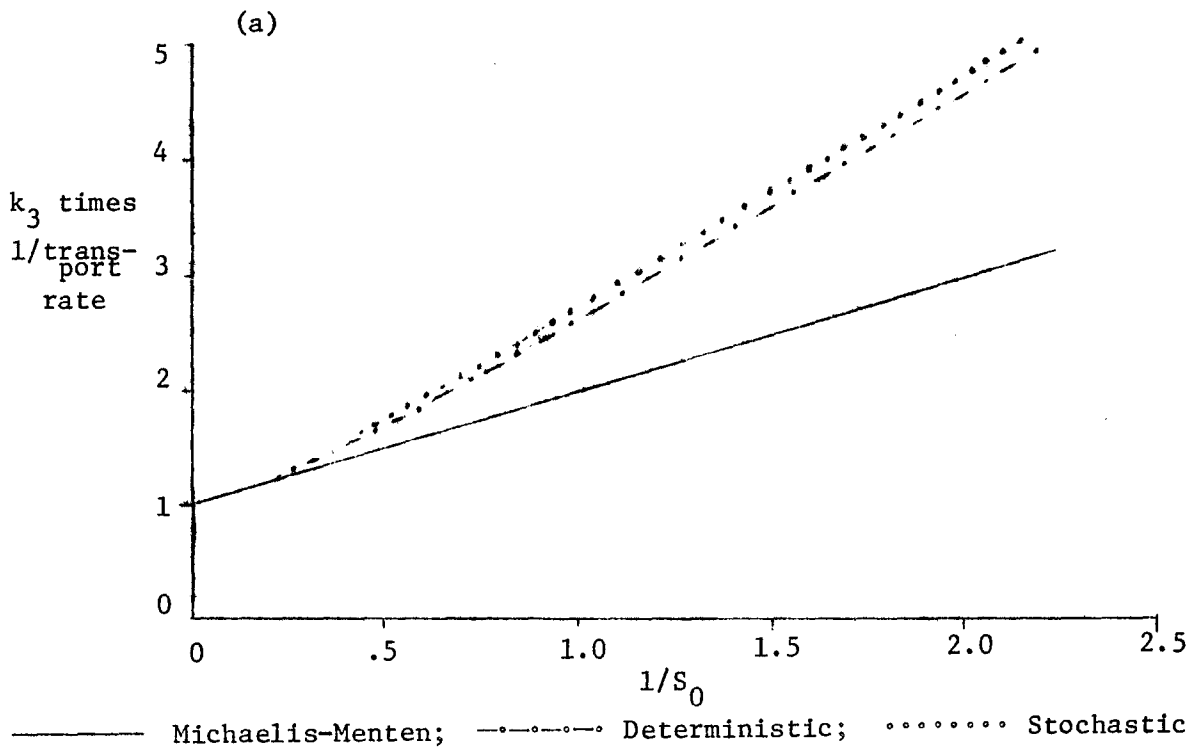
$$\frac{1}{V_s(S_0)} = \frac{1}{k_3}\left[1 - \frac{(1-\rho)k_3}{k_2\rho+k_3+\mu}\right] + A\,\frac{1}{S_0} + o(1) \tag{12}$$

and

$$\frac{1}{V_d(S_0)} = \frac{1}{k_3}\left[1 - \frac{(1-\rho)k_3}{k_2\rho+k_3}\right] + A\,\frac{1}{S_0} + o(1) \tag{13}$$

where $\lim_{S_0\to 0} o(1) = 0$ and the slope of both lines is

$$A = \frac{k_2\mu+k_3(\mu+k_1)}{\lambda k_1 k_3}\,. \tag{14}$$



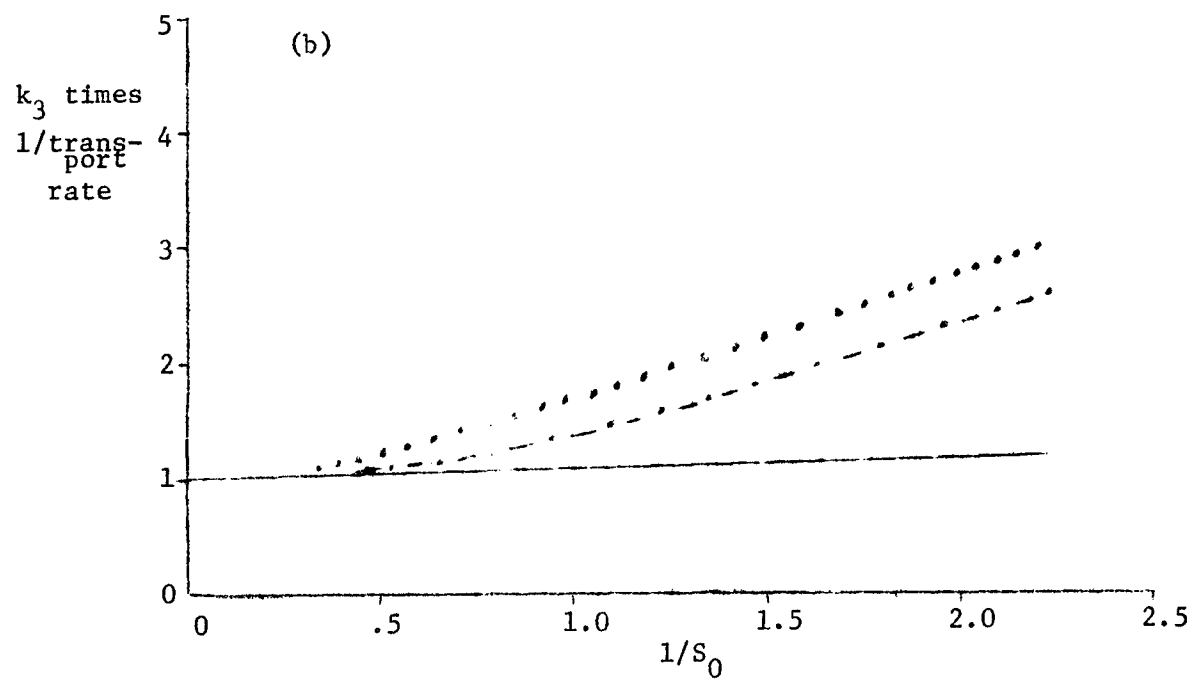


FIGURE 2: Lineweaver-Burke Plots for 3 Models; in (a) $\lambda = \mu = k_3 = 10^2$, $k_1 = k_2 = 10^4$; in (b) $\lambda = \mu = k_3 = 10^2$, $k_2 = 10^4$, $k_1 = 10^5$.

These two lines can be compared with the exact equation for the Michaelis-Menten model

$$\frac{1}{V_m(S_0)} = \frac{1}{k_3} + \frac{K}{k_3}\frac{1}{S_0} \tag{15}$$

where K is defined in equation (8).

Using the asymptotic results given in (12) and (13) one can investigate the properties of the models. For situations in which $\lambda = \mu$ (partition coefficient equal to unity), we see that in the Lineweaver-Burke plot the deterministic and stochastic slopes are always larger than in the Michaelis-Menten model, since $A > K/k_3$ for $\lambda = \mu$. Again when $\lambda = \mu$ and as $\lambda,\mu \to \infty$, $A \to K/k_3$; in addition, the intercepts given in (12) and (13) of the stochastic and deterministic models approach $1/k_3$. Under these conditions the kinetics of the three models are nearly identical.

The intercepts of the asymptotic lines, (12) and (13), are always greater in the stochastic case than in the deterministic case. This implies that for small S_0, $V_s(S_0) < V_d(S_0)$. From our extensive numerical investigation, it seems safe to conjecture that $V_s(S_0) < V_d(S_0)$ for all finite values of S_0. However, because of the complexity of the analytic expression for $V_s(S_0)$ we have been unable to prove this in a satisfactory fashion.

CONCLUSION

In the past, there has been considerable controversy concerning the relevance of stochastic models versus deterministic models. As mentioned previously, Heyde and Heyde (1971) have shown that for a one-substrate, one-product enzyme system (having the implicit assumption of homogeneous subvolumes) the stochastic and deterministic models yield essentially the same

results. However, when dealing with systems at the cellular and sub-cellular levels, one frequently encounters the phenomenon of compartmentation alluded to in Section 1. In this paper, we have presented an example of how incorporation of the concept of compartmentation into the formulation of a stochastic model can yield kinetic results different from those of an analogous deterministic model not utilizing that concept. In a similar manner, Stuart and Branscomb (1971) have demonstrated the "small numbers effect" (the effect of small numbers of reacting molecules per cell) and the "heterogeneity effect" (the effect of the variation in numbers of repressors per cell) as they apply to a stochastic model of *in vivo lac* regulation. In that paper, significant differences between their stochastic model and an analogous deterministic model were also found. Thus it would seem that the question of relevance of stochastic models versus deterministic models can not simply be answered in favor of deterministic models. It is quite likely that an answer to that question depends heavily on the nature of, and the complexity of the system being modelled.

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APPENDIX

Equations (4) and (5) with the left hand sides set equal to zero form a system of linear ordinary differential equations in z. We proceed to solve this system by eliminating the function $F_0(z)$ and obtaining a second order differential equation for $F_1(z)$ alone. Adding (4) and (5) and factoring out $z - 1$ we have

$$o = \lambda^* F_0 - (\mu+k_1)F_0' + (\lambda^*+k_2)F_1 - \mu F_1'. \tag{A.1}$$

Multiplying (5) by $(\mu+k_1)/k_1$ and adding the resulting equation to (A.1) we obtain

$$F_0(z) = \left[\frac{\mu(k_2+k_3)}{\lambda^* k_1} - \frac{\mu}{k_1}(z-1) - \frac{(\lambda^* z - k_3)}{\lambda^*}\right] F_1(z) + \frac{\mu}{\lambda^*}\left[\frac{\mu}{k_1}(z-1) + z\right] F_1'(z). \tag{A.2}$$

Differentiating (A.2), we now have expressions for F_0, F_0' in terms of F_1, F_1' and F_1''; substituting into (5) yields

$$\mu(z-\rho)F_1''(z) + [\rho(\lambda^*+B)+(1-\rho)k_3-\lambda^* z(1+\rho)]F_1'(z) - \frac{\lambda^*}{k_1}(B-\lambda^* z)(1-\rho)F_1(z) = 0 \tag{A.3}$$

where

$$B = \lambda^* + \mu + k_1 + k_2 + k_3. \tag{A.4}$$

Using standard transformations for second order equations (Murphy, 1960) we let

$$F_1(z) = \exp\left(\frac{\lambda^*}{\mu}(z-\rho)\right) V(s) \tag{A.5}$$

where

$$s = \frac{\lambda^*}{\mu}(1-\rho)(\rho-z) \quad \text{and} \quad \rho = \frac{\mu}{\mu+k_1}. \tag{A.6}$$

This transformation is chosen so that $V(s)$ will satisfy Kummer's equation for the confluent hypergeometric function. Substituting (A.5) into (A.3) we obtain after considerable algebra

$$sV''(s) + (b-s)V'(s) - aV(s) = 0 \tag{A.7}$$

where

$$b = 1 + [\lambda^*\rho(1-\rho)+k_2\rho+k_3]/\mu \quad \text{and} \quad a = \frac{k_3}{\mu}. \tag{A.8}$$

The general solution for Kummer's equation can be written in the form

$$V(s) = C_1M(a,b,s) + C_2s^{(1-b)}M(1+a-b,\ 2-b,\ s) \tag{A.9}$$

where $M(a,b,s)$ is the confluent hypergeometric function

$$M(a,b,s) = \sum_{n=0}^{\infty} \frac{(a)n}{(b)_n n!} s^n$$

and

$$(a)n = a(a+1) \ldots (a+n-1) \quad \text{with} \quad (a)_0 = 1.$$

The particular solution of (A.7) where $F_1(z)$ is a generating function is obtained by setting $C_2 = 0$. If $C_2 \neq 0$, then $V(0)$ does not converge; this implies that $F_1(z)$ does not converge at $z = \rho$. C_1 is then determined by the condition that $F_0(z) + F_1(z)$ is a probability generating function and thus

$$F_0(1) + F_1(1) = 1. \tag{A.10}$$

Using known identities for the confluent hypergeometric function (Abramowitz and Stegun, 1964; 505) and (A.5), (A.9) and (A.10) we obtain the equation

used to compute the transport rate for the stochastic model

$$F_1(1) = \lambda^*(1-\rho)\left[\lambda^*(1-\rho)+\rho k_2+k_3\,\frac{M(b-a-1,b,r)}{M(b-a,b,r)}\right]^{-1} \tag{A.11}$$

where

$$r = \frac{\lambda^*}{\mu}(1-\rho)^2. \tag{A.12}$$

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