Transport Properties of Single-File Pores with Two Conformational States

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ABSTRACT Complex facilitative membrane transporters of specific ligands may operate via inner channels subject to conformational transitions. To describe some properties of these systems, we introduce here a kinetic model of coupled transport of two species, L and w, through a two-conformational pore. The basic assumptions of the model are: a) single-file of, at most, n molecules inside the channel; b) each pore state is open to one of the compartments only; c) there is at most only one vacancy per pore; d) inside the channel, a molecule of L occupies the same positions as a molecule of w; and e) there is at most only one molecule of L per pore. We develop a general representation of the kinetic diagram of the model that is formally similar to the one used to describe one-vacancy transport through a one-conformational single-file pore. In many cases of biological importance, L could be a hydrophilic (ionic or nonionic) ligand and w could be water. The model also finds application to describe solute (w) transport under saturation conditions. In this latter case, L would be another solute, or a tracer of w. We derive steady-state expressions for the fluxes of L and w, and for the permeability coefficients. The main results obtained from the analysis of the model are the following. 1) Under the condition of equilibrium of w, the expression derived for the flux of L is formally indistinguishable from the one obtainable from a standard four-state model of ligand transport mediated by a two-conformational transporter. 2) When L is a tracer of w, we can derive an expression for the ratio between the main isotope and tracer permeability coefficients (P_w/P_d). We find that the near-equilibrium permeability ratio satisfies $(n-1) \leq (P_w/P_d)_{eq} \leq n$, a result previously derived for the one-conformational, single-file pore for the case that $n \geq 2$. 3) The kinetic model studied here represents a generalization of the carrier concept. In fact, for the case that $n = 1$ (corresponding to the classical single-occupancy carrier), the near-equilibrium permeability ratio satisfies $0 \leq (P_w/P_d)_{eq} \leq 1$, which is characteristic of a carrier performing exchange-diffusion.

INTRODUCTION

Facilitative transporters present in biological membranes are considered to be integral membrane proteins that operate via conformational transitions between states. A classical model describes them as having the binding site for the transported ligand alternatingly facing the compartments on either side of the membrane (Stein, 1986). Fig. 1 *A* shows the minimum four-state model that describes such processes. This basic model or its modifications have been employed to account for the properties of many transporters of ionic and nonionic ligands (see, for instance, Stein, 1986; Andersen, 1989; Krupka, 1989, 1990; Daumas and Andersen, 1993; Denner et al., 1993) and of active ionic transport systems of the type of electrogenic enzymes (Läuger, 1980, 1984, 1991; Hansen et al., 1981).

The model in Fig. 1 also represents the classical kinetic description of carrier-mediated transport (Schultz, 1980; Stein, 1986). Thus, the term "carrier" has been adopted to account for the transport processes mediated either by translatory molecules of the valinomycin type or by membrane proteins undergoing conformational transitions (Läuger, 1980, 1984). In both cases, the main basic assumption implicit in the four-state scheme of Fig. 1 is that the transporter offers a single binding site to the ligand, accessible from only one side of the membrane at a time (Schultz, 1980). Although this scheme could constitute an accurate description of facilitative transport mediated by lipid-soluble translatory carriers, it might not correspond with the actual mechanisms taking place in complex membrane proteins. In this case, ligand movement through an inner protein channel seems more realistic. Channel-like mechanisms have been suggested to operate, for instance, in mediated ionic transport (Fröhlich, 1988; Krupka, 1989; Hasegawa et al., 1992), in electrogenic membrane systems (Andersen et al., 1985; Lagnado et al., 1988; Nakamoto et al., 1989; Hilgemann et al., 1991; Läuger, 1991; Gadsby et al., 1993; Rakowski, 1993) and in transporters of larger molecules, like choline (Krupka and Devés, 1988), and particularly in facilitative sugar transporters (Barnett et al., 1975; Lowe and Walmsley, 1986; Walmsley, 1988; for a recent review see Baldwin, 1993). In this latter example, kinetic and structural evidence (Mueckler et al., 1985; Jung et al., 1986; Alvarez et al., 1987) and data on water permeability (Fischbarg et al., 1990; Zhang et al., 1991) are suggestive of the existence of a hydrophilic channel accessible both to sugar substrates and water molecules. Similarly, urea and water appear to traverse a channel through the Cystic Fibrosis transmembrane conductance regulator protein (Hasegawa et al., 1992).

From the above, several transporters appear to operate via complex inner channels, subject to conformational transitions and also accessible to water molecules. It seems reasonable to assume that this concept can be generalized to

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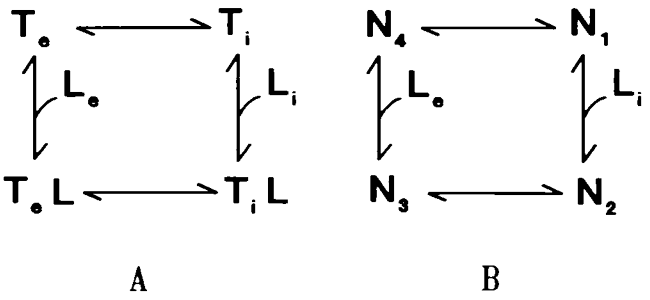

FIGURE 1 The four-state model of ligand transport mediated by a two-conformational transporter. (*A*) The transporter exhibits a binding site either to compartment *e* (form T_e) or to compartment *i* (form T_i). T_eL, T_iL: the ligand-binding states, with the binding sites facing compartments *e* and *i*, respectively. (*B*) The equivalent description used to derive a steady-state expression for the ligand flux in Appendix 1.

most transporters. We analyze here a kinetic model of facilitative transport of two species (w and L) through a common two-conformational channel. We elect to treat the simple case of a single-file channel. Species w is present in nearly saturating activities, whereas species L is present in small activities in the two compartments. In many biological cases, w could be water, whereas L would be a specific ionic or nonionic ligand. Because our purpose is to understand some basic kinetic properties, we do not consider here the effects of transmembrane electric fields.

In the first part, we introduce a procedure to lump transitional steps into overall diagram components, thus simplifying the diagrammatic analysis of the model. We then show the general steady-state expressions derived for the species fluxes and suggest possible applications of the model. To perform the analysis, we employ the King-Altman method (King and Altman, 1956) as modified by Hill (1977).

In the second part, we discuss the conditions under which the present single-file channel model behaves as a four-state model for the transport of L (like the one in Fig. 1). Crucially, we show that when w has equal activities in the two compartments (equilibrium condition), the expression for the steady-state flux of L is identical to that derived from the four-state model of Fig. 1 (Appendix 1; see also Stein, 1986).

In the third part, we derive explicit expressions of the permeability coefficients for w and L. In particular, when L is a tracer of w, we show that the near-equilibrium permeability ratio $(P_w/P_d)_{eq}$ (equivalent to the Ussing coefficient (Hille and Schwartz, 1978; Kohler and Heckmann, 1979)) has lower and upper limits of $(n - 1)$ and n, respectively, for any value of n ($n \geq 1$). These limits are analogous to those arising from the analysis of the one-conformational one-vacancy single-file pore (Kohler and Heckmann, 1979; Finkelstein, 1987, pp. 52–55; Hernández and Fischbarg, 1992), valid for $n \geq 2$. When $n = 1$, the single-file channel model analyzed here becomes the classical carrier performing exchange-diffusion, characterized by a near-equilibrium permeability ratio obeying $0 \leq (P_w/P_d)_{eq} \leq 1$.

THE KINETIC MODEL

We assume that the transport of w across a membrane separating compartments e and i takes place through a number N of identical single-file pores that exist in two conformational states. When saturated, the pores contain a single file of n molecules of w. The molecules occupy specific positions inside the pore, numbered $1, 2, \cdots, n$. Each conformational state exposes a binding site to one of the compartments, while simultaneously closing the access to a binding site on the other compartment. To account for the transport process, we assume that vacancies are generated at the extreme positions of the pore (positions 1 and n), as a consequence of the release of molecules to the compartments. We consider here the case that, at most, only one vacancy exists per pore. For the case of water, this assumption may be justified by the large water activity of biological compartments (Kohler and Heckmann, 1980). The one-vacancy mechanism has been previously adopted to account for the single-file transport process in near-saturation conditions in ionic and water channels (Kohler and Heckmann, 1979; Schumaker and MacKinnon, 1990; Hernández and Fischbarg, 1992).

The pore is also capable of binding another ligand L reversibly to any of the inner positions of the pore. For the purpose of this paper, we assume that a molecule of L occupies the same positions inside the pore as a molecule of w. This assumption implies that, similarly to the molecules of w, L is also restricted by the "no-pass" condition. No further structural restriction is imposed by this assumption, because the binding of L to a particular position is, in general, determined by different rate constants than the ones corresponding to the binding of w to the same position. At a particular position, w and L could bind to different chemical groups present at the channel walls. We also assume that there is at most only one molecule of L per pore. This assumption is valid for sufficiently small activities of L in the compartments. As can be seen (Fig. 4), when $n = 1$ the single-file channel model becomes the classical six-state kinetic model of countertransport (Stein, 1986).

In Fig. 2, we show the kinetic diagram of the model for the case that $n = 3$. Because the number of states equals $2(1 + n + n^2)$, the steady-state kinetic analysis becomes very much involved for larger n s. However, the diagram of Fig. 2 is suggestive of a general form of representation of the process, which permits us to derive some results without performing a detailed kinetic analysis. This general representation is shown in Fig. 3 *A*. The parts of the diagram containing the vacancy states have been collected into "components," called C_w for the case that only molecules of w are present, and C_L^i (with $i = 1, 2, \cdots, n - 1$) for the case that a molecule of L is also present. The superindex "*i*" indicates the position from which L is moved in the *e* to *i* direction when the corresponding component is traversed. Notice that, except for the case that $n = 1$, each component may be traversed through several different trajectories. In general, the component's state having the vacancy exposed to the *i* compartment is called state "E," independently of the particular component. Analogously, the opposite state is

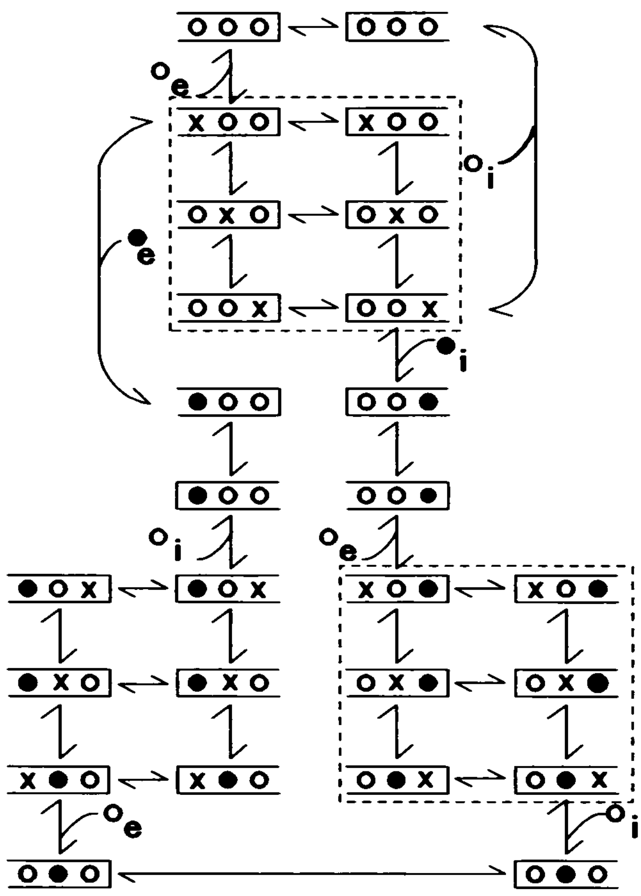

FIGURE 2 The complete kinetic diagram of the model for the case that $n = 3$. (○) Molecules of w; (●) Molecules of L; (×) Vacancies. Components C_w and C_L^2 are enclosed by dashed rectangles.

called "I". In the *e* to *i* direction, components are traversed from state E towards state I.

The general diagram of Fig. 3 *A* resembles the kinetic description of the one-vacancy, single-file transport process through a one-conformational pore (Kohler and Heckmann, 1979; Hernández and Fischbarg, 1992). The difference relates to the presence of the conformational transition steps. Hence, the "components," containing the conformational transitions between vacancy states, substitute the linear sequences of steps of "jumping" between sequential positions of the one-conformational pore model. Also, conformational transitions between saturated states appear in the linear portions of the diagram. However, the steps corresponding to the release and binding of L and w maintain their relative positions. As a consequence of the similarity between the general kinetic diagrams involved, some of the results obtained here are analogous to those previously obtained by us from the kinetic analysis of a one-conformational pore model of water transport (Hernández and Fischbarg, 1992).

The diagram of Fig. 3 *A* can be used with advantage to perform the steady-state kinetic analysis of the process. We use the diagrammatic formalism developed by Hill (1977) to derive steady-state expressions for the fluxes of L and w. To perform the analysis, we distinguish the three "general" cycles contained in the diagram of Fig. 3*A*. These three cycles are called "a," "b," and "c" and are shown in Fig. 3*B*. In Appendix 2, we derive the general steady-state expressions of the model.

From (A5), we express the molar flux of w for $L_e = L_i = 0$, taken positive in the *e* to *i* direction, by (A6) as

$$J_w = \frac{(N)\Sigma_b E_i' P_i}{E'}(w_e - w_i). \tag{1}$$

Therefore, the permeability coefficient P_w of w is given by

$$P_w = \frac{(N)\Sigma_b E_i' P_i}{E'}. \tag{2}$$

For the case that w is water, and considering the assumptions of the model, we can identify P_w with the osmotic permeability coefficient P_f of the membrane (or at least, with the contribution of the system analyzed here to the total permeability):

$$P_w = P_f. \tag{3}$$

Because E' is a function of the activities of w in the compartments, in this case P_w depends upon these activities. As discussed previously (Hernández and Fischbarg, 1992), this is a consequence of the vacancy mechanism responsible of the transport process, and it constitutes a typical property of discontinuous diffusion (Schultz, 1980).

The molar flux of L is given by (A9):

$$J_L = (N)[\Sigma_a E_k P_k (w_e^{n-1} w_i L_e - w_e w_i^{n-1} L_i) + \Sigma_c E_j P_j (w_e^{n-1} L_e - w_i^{n-1} L_i)]/E_T. \tag{4}$$

When $w_e = w_i = w$, we obtain from (A12) the permeability coefficient of L as a function of w and of the activities of L:

$$(P_L)_w = \frac{(N)\,(w^n \Sigma_a E_k P_k + w^{n-1} \Sigma_c E_j P_j)}{(E_T)_w}. \tag{5}$$

From (4) we notice that the model implies that a nonzero flux J_L may be obtained for equilibrium activities of L, that is for $L_e = L_i$, provided that w_e and w_i are different. This is a consequence of the flux coupling between both species implied in the model. In particular, this property provides us with the possibility of experimentally testing models where w (e.g., water) is also a ligand of the solute (L) transporter.

There are two main situations of biological importance where the model analyzed here may find application: 1) facilitative transport of a solute (L) through a path also accessible to water molecules (w), and 2) transport of an ionic or nonionic ligand (w) present in saturating activities. In this latter case, L could be another ligand transported by the system and present in sufficiently small activities or a tracer of w. In both situations, the accomplishment of the "no-pass" (or single-file) condition is required.

In the section "Validity of the Four-State Model" we analyze the conditions under which the general model shown in Fig. 3*A* exhibits the macroscopic behavior of a four-state model used to represent the transport of L, similar to the

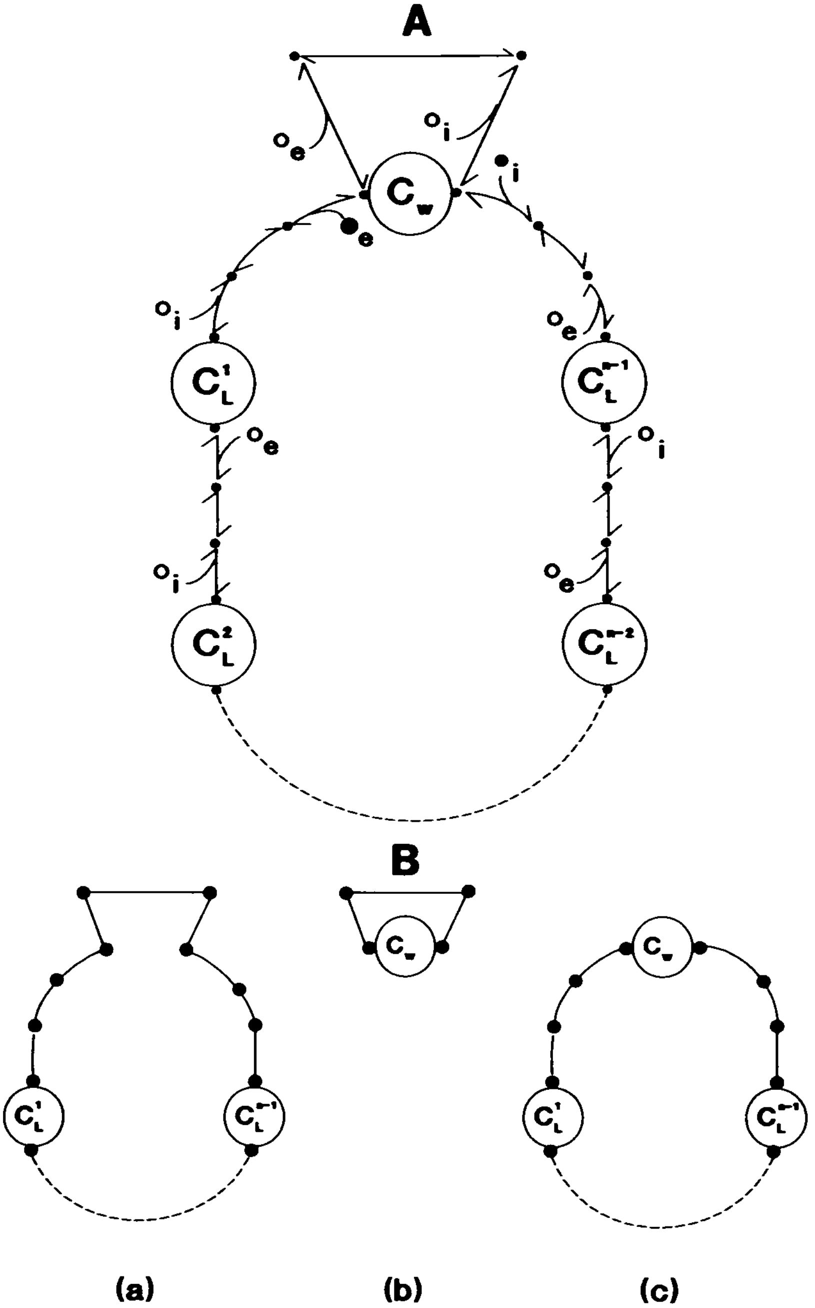

FIGURE 3 (*A*) The general kinetic diagram of the model. The small black circles represent pore states. C_w is a "component" containing vacancy states having water molecules only; $C_L^1, \cdots, C_Ln$-1 are "components" containing vacancy states having the ligand molecule (see text for further details). (*solid lines*) Actual individual transitions; (*broken line*) several intermediate steps, consisting of a repeated sequence of components C_L^i and transitions. (*B*) The three general cycles comprising the general diagram of *A*. The symbols employed here have the same meanings as in that figure. See the text for the meaning of the general cycles.

one shown in Fig. 1. Later, in the section "Permeability Coefficients," we use the general expressions shown above to analyze a more particular situation and also to discuss the case where L is a tracer of w.

VALIDITY OF THE FOUR-STATE MODEL

If $n = 1$ and only one ligand is present (either w or L), the four-state model of Fig. 1 applies. For larger *n*s, and when only w is present, a four-state model is still valid as an approximation, under conditions of the parameters that permit to collapse the transitions connecting the states having the vacancy in positions 1 and *n*, into a single transitional step. We now analyze whether the model may exhibit a four-state diagram behavior for the transport of L, for the case that $n \geq 1$ and when both w and L are present.

Under certain necessary conditions, a kinetic diagram may be reduced to a diagram consisting of a lower number of states (Hill, 1977). For the case analyzed here, one possibility is to consider that the vacancy states are transient intermediates and, therefore, can be eliminated from the diagram. The reduced diagram now includes some of the original transition steps with their original rate constants and also the reduced transitional steps corresponding to the "reduced" rate constants. These reduced rate constants are functions of the original ones. In general, under this hypothesis, the number of states of the reduced diagram equals $2(n + 1)$. Therefore, only in the case that $n = 1$ we obtain a four-state reduced diagram (Fig. 4). In Appendix 3, we perform the model reduction for the case that $n = 1$ and assuming that the vacancy states are transient intermediates. As it is shown there, the necessary condition for reduction ((A17)) may be accomplished for large activities of w in the compartments.

Inspection of Fig. 4 *B* shows that the reduced model for $n = 1$ becomes similar to the four-state model of Fig. 1 if the reduced rate constants r_{14}, r_{41}, r_{36}, and r_{63} are nil (or negligible). From (A19) we see that if $t_{ei} = t_{ie} = 0$, then $r_{14} = r_{41} = r_{16} = r_{61} = r_{34} = r_{43} = r_{36} = r_{63} = 0$. However, this condition does not make the reduced rate constants r_{13}, r_{31}, r_{46}, and r_{64} nil. Hence, in this particular example, because the original rate constants u_{ei}, u_{ie}, s_{ei}, and s_{ie} are not affected by the reduction procedure, the model in Fig. 4 *B* becomes similar to the model in Fig. 1. Therefore, for the hypothesis assumed, only for the case that $n = 1$, and under a severely restrictive condition, the model introduced here may be reduced to the four-state model of solute transport. We may conclude that this situation is extremely improbable, and that the four-state reduced model does not represent a relevant particular case of the model of a complex transporter analyzed here.

Although, from the above, we cannot justify the standard four-state model as a particular case of a general model of transport of w and L in complex membrane systems, we may ask ourselves whether the model introduced in this article is able to exhibit kinetic properties not distinguishable from those of a four-state model under some circumstances. Indeed, when $w_e = w_i = w$, the flux of L is given by (A12):

$$(J_L)_w = \frac{(N)\,(w^n\Sigma_a E_k P_k + w^{n-1}\Sigma_c E_j P_j)}{(E_T)_w}\,(L_e - L_i). \qquad (6)$$

In this case ($w_e = w_i = w =$ constant), every binding step involving molecules of w will be characterized by a pseudo-first-order rate constant β_j, given by

$$\beta_j = b_j w, \qquad (7)$$

where b_j is the second-order rate constant of binding of step j.

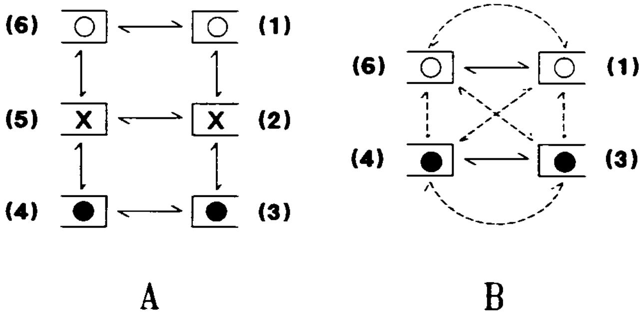

FIGURE 4 Kinetic diagram of the model for the case that $n = 1$. (*A*) The kinetic diagram including all the states. The numbering of the states is used to perform the model reduction in Appendix 3. (*B*) The corresponding reduced model, under the assumption that the vacancy states are transient intermediates. The solid lines represent the actual original transitions, the broken lines are "reduced" transitions, characterized by "reduced" rate constants (see text).

Under these conditions, the directional diagrams of the model in Fig. 4 fall into four groups, those having as common factors L_eL_i, L_e, and L_i, and those independent of the activities of L (Fig. 5). Therefore, the denominator $(E_T)_w$ is of the form

$$(E_T)_w = R_1L_eL_i + R_2L_e + R_3L_i + R_4, \qquad (8)$$

where the coefficients $R_1, \cdots, R_4$ are functions of w and of the rate constants.

Comparison of (6) and (8) with (A1)–(A3) shows that, under the condition of equilibrium of w between the compartments, the flux $(J_L)_w$ is given by an expression formally analogous to the one derived for the four-state model of Fig. 1. If the model analyzed here represents an actual transport process, the coefficients of expressions of the form of (8) or (A3) should be functions of the equilibrium activity w. In particular, we may conclude that, for a transporter described by a solute-water (L-w) model having similar properties to the one introduced here, kinetic experiments of ligand transport performed at a zero or very small water activity difference between compartments can be interpreted in terms of a simple four-state solute model. However, this four-state model will not provide with an accurate mechanistic description of the transport process.

Other particular situations simulating somehow a four-state model behavior may occur (for instance, involving particular values of the intermediate rate constants), but they are not analyzed here.

PERMEABILITY COEFFICIENTS

From (A5), we notice that the flux of w is partially coupled to the transport of L. If the activities of L are sufficiently small, the contribution of the coupling terms to the flux of w may be negligible. Hence, we assume that the permeability coefficient of w is, for all practical purposes, given by expression (2), which was obtained under the condition that $L_e = L_i = 0$. When $w_e \approx w_i = w$, we obtain the near-equilibrium permeability coefficient $(P_w)_{eq}$:

$$(P_w)_{eq} = \frac{(N)\Sigma_b E'_i P_i}{E'_w}, \qquad (9)$$

where E'_w is a function of w.

From (2), (3), and (9) we see that the effective ratio $P_w/(P_w)_{eq}$ is a function of w_e, w_i, and of the particular equilibrium activity w,

$$P_w/(P_w)_{eq} = E'_w/E', \qquad (10)$$

and can be smaller or larger than one.

The general expression for the flux of L in the model is given by (A9) (or Eq. 4). If the activities of L are sufficiently small, to account for the following condition:

$$b_{L,e}L_e < r_{L,e} \quad \text{and} \quad b_{L,i}L_i < r_{L,i}, \qquad (11)$$

then E_T is given by

$$E_T = E'\,E_L, \qquad (12)$$

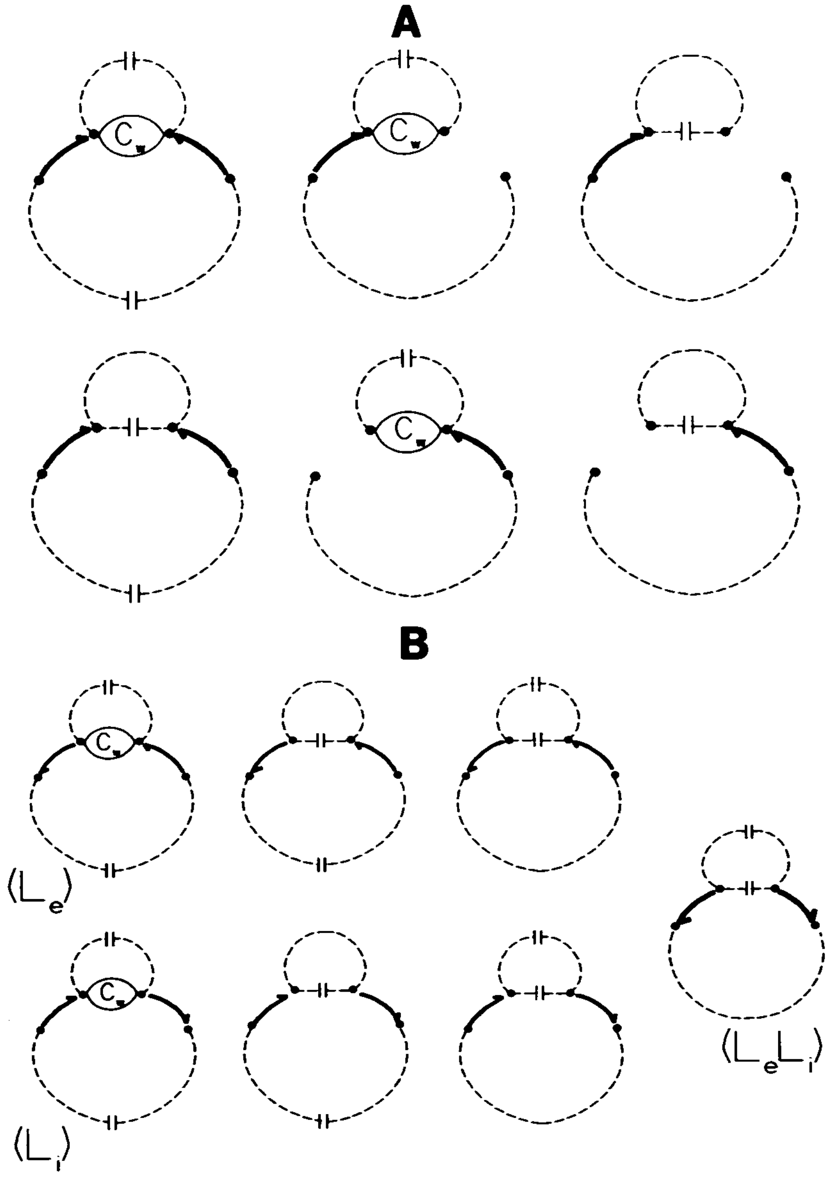

FIGURE 5 General representations of the directional diagrams for the case of equal water activities in the two compartments. The symbols have the same meaning as in Fig. 3. The symbol (‖) represents a complete interruption of the diagram. In the linear segments, such as interruption occurs when a single transitional step is absent, in the components it occurs by interrupting all the possible trajectories between states E and I by extracting the necessary minimum number of transitional steps. (*A*) General schemes of the directional diagrams independent of the ligand activities. (*B*) Analogous schemes for directional diagrams containing L_eL_i, L_e, and L_i as common factors.

where E_L is the sum of all the parts of the flux diagrams that feed into cycle b and is a function of the activities of w. For the case that $w_e = w_i = w$,

$$(E_T)_w = E'_w\,(E_L)_w. \tag{13}$$

Under condition (13), expression (5) becomes

$$(P_L)_w = \frac{(N)\,(w^n\Sigma_a\,E_kP_k + w^{n-1}\,\Sigma_cE_jP_j)}{E'_w\,(E_L)_w} \tag{14}$$

From (9) and (14), we express

$$(P_L)_w = (P_w)_{eq}\,\frac{(w^n\Sigma_a\,E_kP_k + w^{n-1}\,\Sigma_c\,E_jP_j)}{(E_L)_w\Sigma_b\,E'_iP_i}. \tag{15}$$

If L is a tracer of w having diffusive properties identical to those of common w, we can identify $(P_L)_w$ with the diffusive permeability of w, P_d:

$$(P_L)_w = P_d. \tag{16}$$

In this case, the following identities between the rate constants and component terms occur (see Appendix 2):

$$\begin{aligned} &b_{w,e} = b_{L,e};\quad b_{w,i} = b_{L,i};\quad r_{w,e} = r_{L,e};\\ &r_{w,i} = r_{L,i};\quad s_{ei} = u_{ei};\quad s_{ie} = u_{ie};\\ &C_{w,ei} = C^i_{L,ei}\quad (\text{for all } i = 1, 2, \cdots, n-1);\\ &C_{w,ie} = C^i_{L,ie}\quad (\text{for all } i = 1, 2, \cdots, n-1); \end{aligned} \tag{17}$$

and

$$D_w = D^i_L \qquad (\text{for all } i = 1, 2, \cdots, n-1).$$

The terms D^i_L represent sums analogous to D_w (see Appendix 2), provided by the particular components C^i_L.

Under (17), $(E_L)_w$ is given by

$$(E_L)_w = n\,(\Pi_w\,w)^{n-1}\,Q + (n-1)\,(\Pi_w)^{n-2}\,w^nS, \tag{18}$$

where

$$Q = s_{\mathrm{ic}} r_{\mathrm{w,c}} + s_{\mathrm{ci}} r_{\mathrm{w,i}} + r_{\mathrm{w,c}} r_{\mathrm{w,i}},$$

and (19)

$$S = b_{\mathrm{w,c}}\, b_{\mathrm{w,i}} s_{\mathrm{ic}}\, s_{\mathrm{ci}} r_{\mathrm{w,c}}\, r_{\mathrm{w,i}} D_{\mathrm{w}},$$

and where Π_{w} is defined by (A7).

Using expressions (A7), (A8), (A10), (A11), (18), and (19), and conditions (16) and (17), we obtain from (15) the near-equilibrium ratio of the permeability coefficients of *w*:

$$\frac{(P_{\mathrm{w}})_{\mathrm{eq}}}{P_{\mathrm{d}}} = \frac{n\Pi_{\mathrm{w}} Q + (n-1)wS}{\Pi_{\mathrm{w}} Q + wS}. \tag{20}$$

For the case of water, this expression gives the ratio between the osmotic and diffusive permeability coefficients, $(P_{\mathrm{f}})_{\mathrm{eq}}/P_{\mathrm{d}}$.

From the inspection of Eq. 20, we see that $(n - 1) \leq (P_{\mathrm{w}})_{\mathrm{eq}}/P_{\mathrm{d}} \leq n$, a result encountered for the one-conformational single-file pore (Kohler and Heckmann, 1979; Hernández and Fischbarg, 1992) for the case that $n \geq 2$ (for the case that $n = 1$, the one-conformational single-file pore has a permeability ratio that always equals one). As already commented, this result is a consequence of the formal similarity between the diagram of Fig. 3*A* and the diagrams used by those authors to describe single-file transport through the one-conformational pore. From (10) and (20), we realize that, depending on the activities of w, the ratio $P_{\mathrm{w}}/P_{\mathrm{d}}$ can be larger than *n*, a property attributable to the discrete nature of the kinetic process and already found for the case of the one-conformational single-file pore (Hernández and Fischbarg, 1992).

As already mentioned (see "Validity of the Four-State Model"), for the case that $n = 1$, the model analyzed here becomes the classical description of a single-occupancy carrier able to perform countertransport of two different ligands, w and L (see Fig. 4 *A*). Therefore, in this case, if L is a tracer of w, the near-equilibrium permeability ratio (Eq. 20) satisfies $0 \leq (P_{\mathrm{w}})_{\mathrm{eq}}/P_{\mathrm{d}} \leq 1$, a result already derived for carriers performing "exchange diffusion" (Läuger, 1980; Schultz, 1980). Hence, the classical carrier constitutes a particular kinetic case (the case that $n = 1$) of the two-conformational multi-occupancy channel analyzed here. As previously discussed by Läuger (1980, 1984), "carrier-like" behaviors occur as limiting cases of single-occupancy channels with many conformational states, in the presence of high activation energy barriers for the binding steps, that determine the condition of being "closed" on one side of the pore at a given time.

DISCUSSION

The model introduced in this article represents an example of the kinetic description of coupled transport of two species through a two-conformational transporter. As already mentioned, the purpose of this article has been to provide with some basic kinetic aspects of a plausible mechanism of transport through complex membrane proteins and not to modelize a particular case of mediated transport. As it is, the model analyzed here could constitute, for instance, a basis to represent single-occupancy transport of inorganic ions through an aqueous channel undergoing conformational transitions. In this case, the application of the model requires the introduction of the electrical effects on the rate constants, a problem under discussion (see, for instance, Cooper et al., 1988; Dani and Levitt, 1990). As already suggested here, the model could also be employed to represent ionic transport under near-saturation conditions. In this case, w would be the saturating ion, and L would be another ion present in low activities, or a tracer of w. As in the previous case, the electrical effects should be introduced. Concerning the possible application of the model to transporters of hydrophilic ligands of larger size, such as amino acids or sugars, we offer the following speculations. Of all the assumptions of the model, the fact that each conformational state is open at any given time to only one of the compartments seems a plausible property of this type of transporters. Although it is possible, for instance, that the glucose transporters operate by means of an inner hydrophilic channel, there is no evidence available to support the idea of a single-file of water molecules inside it. In fact, considering the dimensions of the glucose molecule obtainable either from a graphic simulation (approx. 9.2 × 6.0 × 5.2 Å in Van der Waals size) or from diffusion coefficient measurements (Longsworth, 1953; Stokes-Einstein radius: ~3.2 Å), compared with the dimensions of the water molecule (~1.4 Å Van der Waals radius), one would expect more than one file of water molecules inside such a channel. In this type of situation, schemes still more complicated than the one analyzed here should be developed to represent the transport processes.

One of the main conclusions of the analysis presented here is that, if a given solute transporter is also able to bind water molecules in the same path available for the solute, the models developed to describe the process need to include water as a second ligand. In this connection, under the condition of water equilibrium, the kinetic expression derived here for the flux of ligand (L) (Eqs. 6–8) is formally indistinguishable from the one obtainable for the minimum four-state model of solute transport through a two-conformational transporter (Eqs. A1–A3). If this standard four-state model description is adopted, and the transporter is actually performing transport of both solute and water, the rate constants of the four-state model will be misinterpreted as true first-order rate constants independent of the water activities, whereas they could actually correspond to expressions of the rate constants of a more complicated diagram involving water as a ligand. It is interesting to note that, because of the similarity of diagrams commented below, an analogous conclusion could be reached from the analysis of the transport process of a ligand L mediated by a one-conformational, single-file pore under equilibrium saturating activities of w (Hernández and Fischbarg, 1990). These results suggest that the analysis of kinetic data about species fluxes is not sufficient to distinguish between a simple four-state-carrier mediated transport and more complicated mechanisms, such as one-conformational or two-conformational single file pores.

The general scheme developed here to represent the transport process through the two-conformational single-file pore is formally similar to the one employed in the case of the one-conformational single-file pore. As a result of this similarity, the kinetic expressions derived here for the permeability coefficients are formally analogous to those obtainable from the analysis of the one-conformational single-file pore under near-saturation conditions (Kohler and Heckmann, 1979; Hernández and Fischbarg, 1992). Correspondingly, we also derive here the noteworthy result that, analogously to the one-conformational single-file pore with $n \geq 2$, the near-equilibrium ratio between the main and tracer permeability coefficients of the saturating species w satisfies $(n - 1) \leq (P_w)_{eq}/P_d \leq n$.

An important conclusion of the present study is that the model analyzed here represents a generalization of a carrier system, and that the classical carrier able to perform countertransport of two different species, and to exhibit exchange-diffusion of the main and tracer isotopes of a single species, constitutes a particular case of the two-conformational pore. This result contributes to the concept that classical carriers and channels are particular cases of more general types of transport systems, an idea previously advanced by Läuger (1980, 1984, 1987) and confirmed for the case of ionic transport by using physical models (Berry and Edmonds, 1992, 1993) and by a theory combining the Poisson and Nernst-Planck equations (Chen and Eisenberg, 1993).

Concerning the possible application of some of these results to the problem of water transport, it has been demonstrated that part of the water movement across biological membranes takes place through diverse protein channels or transporters. Most of such movement takes place across channels specific for water (e.g., the CHIP28 water channel protein (Preston et al., 1992); the WCH-CD water channel protein (Fushimi et al., 1992); the CHIP28k water channel protein (Zhang et al., 1993); the tonoplast intrinsic protein (Maurel et al., 1993), but some movement also takes place across other membrane proteins (the nicotinic acetylcholine receptor channel (Dani, 1989); glucose transporters (Fischbarg et al., 1990; Zhang et al., 1991); the CFTR (Hasegawa et al., 1992)). It might be necessary to employ kinetic analysis to interpret the permeability properties of such complex transporters. A detailed kinetic analysis of the processes mediated by these systems can become rather involved. On the other hand, it is possible that the use of general techniques such as the diagrammatic method employed here will result sufficient to understand some basic aspects of solute and water transport. Our use in this article of the algorithm developed by Hill (1977) to obtain conclusions from the analysis of the simplified scheme of Fig. 3 constitutes a successful example of this type of approach.

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APPENDIX 1

Steady-state solution of the four-state model

We derive here a steady-state expression of the ligand flux for the model of Fig. 1 using, as in the rest of the article, the diagrammatic method developed by Hill (1977). Our purpose is to compare this expression with that obtained in the section "Validity of the Four-State Model." Other analysis of this model have already been performed (see, for instance, Stein, 1986).

For the analysis, we refer to Fig. 1 *B*, where $N_1, \cdots, N_4$ represent the corresponding states of Fig. 1 *A*. We assume the conservation condition, that is, that the total number of transporters (or moles) per unit area (N) remains constant: $(N) = (N_1) + \cdots + (N_4) = \text{constant}$, where (N_i) designs the number of transporters (or moles) per unit area in state N_i. The rate constant k_{ij} determines the transition between states N_i and N_j in the *i* to *j* direction, and k_{ji} determines the transition in the opposite, *j* to *i*, direction $(i, j = 1, 2, 3, 4)$. Because there is only one cycle, only transitions between sequential states in the cycle are taking place. k_{12} and k_{43} are second-order rate constants; the rest are first-order rate constants. The ligand activities in the compartments are designed by L_e and L_i. The steady-state ligand flux J_L is then given by the cycle flux:

$$J_L = [(N)\Pi/A](L_e - L_i), \tag{A1}$$

where, from the condition of detailed balance,

$$\Pi = k_{12}k_{23}k_{34}k_{41} = k_{14}k_{43}k_{32}k_{21}, \tag{A2}$$

and where A is the sum of all the directional diagrams of all the states of the model. Taking the ligand activities as common factors, we express A by

$$A = A_1L_eL_i + A_2L_e + A_3L_i + A_4, \tag{A3}$$

where

$$\begin{aligned} A_1 &= k_{12}k_{43}(k_{23} + k_{32}),\\ A_2 &= k_{43}[k_{14}(k_{23} + k_{32} + k_{21}) + k_{32}k_{21}],\\ A_3 &= k_{12}[k_{41}(k_{23} + k_{32} + k_{21}) + k_{23}k_{34}] \end{aligned} \tag{A4}$$

and

$$A_4 = k_{41}[k_{34}(k_{23} + k_{21}) + k_{32}k_{21}] + k_{14}[k_{21}(k_{32} + k_{34}) + k_{23}k_{34}].$$

APPENDIX 2

Steady-state solution of the two-conformational single-file pore model

We derive here the general expressions of the steady-state fluxes of L and w for the model of Fig. 4. The following symbols represent: $b_{w,e}$, $b_{w,i}$: rate constants of binding w from compartments *e* and *i*; $r_{w,e}$, $r_{w,i}$: rate constants of release of w to compartments *e* and *i*; $b_{L,e}$, $b_{L,i}$: rate constants of binding L from compartments *e* and *i*; $r_{L,e}$, $r_{L,i}$: rate constants of release of L to compartments *e* and *i*; s_{ei}, s_{ie}: rate constants of the conformational transitions of pores completely filled with molecules of w, "transporting" the open site in the *e* to *i* and *i* to *e* directions, respectively; u_{ei}, u_{ie}: rate constants of the conformational transitions of pores without vacancies and having the ligand L in any position, "transporting" the open site in the *e* to *i* and *i* to *e* directions, respectively; w_e, w_i: activities of w in compartments *e* and *i*; L_e, L_i: activities of L in compartments *e* and *i*; (N): total number of pores per unit area of the membrane.

The application of the diagrammatic method to the scheme of Fig. 4 allows one to derive general expressions for the fluxes of L and w. The flux of w, J_w, is given by

$$J_w = \left[\frac{(N)}{E_T}\right][\Sigma_b E_i P_i (w_e - w_i) + (n-2)\Sigma_a E_k P_k (w_e^{n-1} w_i L_e - w_e w_i^{n-1} L_i) + (n-1)\Sigma_c E_j P_j (w_e^{n-1} L_e - w_i^{n-1} L_i)], \tag{A5}$$

where E_T is the sum of all the directional diagrams of all the states of the model, and where the terms $\Sigma E_h P_h$ represent sums, taken over the general cycles *a*, *b*, and *c* of Fig. 6, of all the cycle fluxes $E_h P_h$ ($h = i, j, k$) determined by each general cycle. In expression A5, the condition of detailed balance has been applied to all the cycle fluxes. Hence, P_h represents the product of the rate constants in any of the directions, for the particular cycle *h*. E_h is the sum of all the parts of the flux diagrams that feed into cycle *h*.

When $L_e = L_i = 0$, the flux of w is determined by the single general cycle *b*:

$$J_w = \frac{(N)\Sigma_b E_i' P_i (w_e - w_i)}{E'}, \tag{A6}$$

where E' is the sum of all the directional diagrams of all the states corresponding to the general cycle *b* only, and where $\Sigma_b E_i' P_i$ is the sum of all the cycle fluxes determined by the general cycle *b*. For the denominator E', the appendages to the cycles only connect the states contained in the general cycle *b* (E_i'). In relation with this, it can be demonstrated from the kinetic analysis that $E_i' = E_i/E_L$ for every cycle *i* in the general cycle *b* (see expression 12).

We define

$$\Pi_w = \Sigma_b E_i' P_i. \tag{A7}$$

From the detailed balance condition,

$$\Pi_w = b_{w,i} s_{ie} r_{w,e} C_{w,ie} = b_{w,e} s_{ci} r_{w,i} C_{w,ci}, \tag{A8}$$

where $C_{w,ci}$ and $C_{w,ie}$ are the sums of all the products of uninterrupted directional trajectories inside C_w and their corresponding appendages feeding into them from all the states inside the component, from the E to I and I to E states, respectively.

The flux of L, J_L, is given by

$$J_L = (N)[\Sigma_a E_k P_k (w_e^{n-1} w_i L_e - w_e w_i^{n-1} L_i) + \Sigma_c E_j P_j (w_e^{n-1} L_e - w_i^{n-1} L_i)]/E_T, \tag{A9}$$

where, from the detailed balance condition,

$$\begin{aligned} \Sigma_a E_k P_k &= b_{L,i} (u_{ie})^n (r_{w,e})^{n-1} (b_{w,i})^{n-1} r_{L,e} C_{L,ie}^1 C_{L,ie}^2 \cdots C_{L,ie}^{n-2} C_{L,ie}^{n-1} b_{w,e} s_{ci} r_{w,i} D_w \\ &= b_{L,e} (u_{ci})^n (r_{w,i})^{n-1} (b_{w,e})^{n-1} r_{L,i} C_{L,ci}^1 C_{L,ci}^2 \cdots C_{L,ci}^{n-2} C_{L,ci}^{n-1} b_{w,i} s_{ie} r_{w,e} D_w \end{aligned} \tag{A10}$$

and

$$\begin{aligned} \Sigma_c E_j P_j &= b_{L,i} (u_{ie})^n (r_{w,e})^{n-1} (b_{w,i})^{n-1} r_{L,e} C_{L,ie}^1 C_{L,ie}^2 \cdots C_{L,ie}^{n-2} C_{L,ie}^{n-1} C_{w,ie} [r_{w,e} r_{w,i} + s_{ci} r_{w,i} + s_{ie} r_{w,e}] \\ &= b_{L,e} (u_{ci})^n (r_{w,i})^{n-1} (b_{w,e})^{n-1} r_{L,i} C_{L,ci}^1 C_{L,ci}^2 \cdots C_{L,ci}^{n-2} C_{L,ci}^{n-1} C_{w,ci} [r_{w,e} r_{w,i} + s_{ci} r_{w,i} + s_{ie} r_{w,e}]. \end{aligned} \tag{A11}$$

The terms $\Sigma_a E_k P_k$ and $\Sigma_c E_j P_j$ are sums, taken over the general cycles *a* and *c*, similar to the sum defined above for the general cycle *b*. The terms $C_{L,ci}^1, \cdots, C_{L,ci}^{n-1}$, and $C_{L,ie}^1, \cdots, C_{L,ie}^{n-1}$ represent sums analogous to the ones defined for component C_w, differing between them as a consequence of the different position of the ligand L inside each component. The term D_w is the sum of all the appendages generated inside C_w, feeding into the general cycle a via states E and I.

When $w_e = w_i = w$, J_L becomes

$$J_L = \frac{(N)(w^n \Sigma_a E_k P_k + w^{n-1} \Sigma_c E_j P_j)}{(E_T)_w} (L_e - L_i), \tag{A12}$$

where $(E_T)_w$ is E_T under the condition of equal activity of w in the compartments.

The expressions derived here are general, except for the fact that the transitional constants u_{ci} and u_{ie} have been considered independent of the position of L inside the pore.

APPENDIX 3

Reduction of the model for the case that $n = 1$

We perform here the reduction of the model in Fig. 3*A*, under the condition that the vacancy states are transient intermediates, and for the case that $n = 1$. The methodology used here follows the one discussed in Hill (1977). For the analysis, we refer to Fig. 4. The intermediate pore states are designed as $N_1, N_2, \cdots, N_6$ according to the numbering of the states shown in that figure. The rate constants t_{ci} and t_{ie} correspond to the conformational transitions of the vacancy-containing pores "transporting" the open site in the *e* to *i* and *i* to *e* directions, respectively.

The necessary condition for reduction is that, under any situation,

$$P(N_2) < P(N_1),\ P(N_3) \quad \text{and} \quad P(N_5) < P(N_4),\ P(N_6), \tag{A13}$$

where $P(X)$ represents the probability (frequency) of state *X* in the pore ensemble.

From condition A13, we assume that states N_2 and N_5 always approximately accomplish

$$d(N_2)/dt = d(N_5)/dt = 0. \tag{A14}$$

From the expressions obtainable for $d(N_2)/dt$ and $d(N_5)/dt$ from the model in Fig. 4 *A*, and after some algebra, condition A14 leads to

$$(N_2) = \frac{[r_{w,i}(N_1) + r_{L,i}(N_3)](t_{ci} + b_{L,e}L_e + b_{w,e}w_e) + t_{ci}[r_{L,e}(N_4) + r_{w,e}(N_6)]}{D}$$

and

$$(N_5) = \frac{[r_{L,e}(N_4) + r_{w,e}(N_6)](t_{ie} + b_{L,i}L_i + b_{w,i}w_i) + t_{ie}[r_{w,i}(N_1) + r_{L,i}(N_3)]}{D}, \tag{A15}$$

where

$$D = t_{ci}(b_{w,i}w_i + b_{L,i}) + t_{ie}(b_{w,e}w_e + b_{L,e}L_e) + (b_{w,i}w_i + b_{L,i}L_i)(b_{w,e}w_e + b_{L,e}L_e). \tag{A16}$$

From (A15) and (A16), we recognize that condition A13 is achieved if, for instance,

$$b_{w,e}w_e,\ b_{w,i}w_i > r_{w,e},\ r_{w,i},\ r_{L,e},\ r_{L,i}. \tag{A17}$$

We proceed through successive substitutions to obtain, from (A15), expressions for $d(N_1)/dt$, $d(N_3)/dt$, $d(N_4)/dt$, and $d(N_6)/dt$ as functions of (N_1), (N_3), (N_4), (N_6), and the rate constants. We show the expressions obtained:

$$\frac{\mathrm{d}(N_1)}{\mathrm{d}t} = [r_{31}w_i(N_3) - r_{13}L_i(N_1)] + [r_{41}w_i(N_4) - r_{14}L_e(N_1)]$$

$$+ [(r_{61}w_i + s_{ei})(N_6) - (r_{16}w_e + s_{ie})(N_1)],$$

$$\frac{\mathrm{d}(N_3)}{\mathrm{d}t} = [r_{13}L_i(N_1) - r_{31}w_i(N_3)] + [r_{63}L_i(N_6) - r_{36}w_e(N_3)]$$

$$+ [(r_{43}L_i + u_{ei})(N_4) - (r_{34}L_e + u_{ie})(N_3)],$$

$$\frac{\mathrm{d}(N_4)}{\mathrm{d}t} = [r_{14}L_e(N_1) - r_{41}w_i(N_4)] + [r_{64}L_e(N_6) - r_{46}w_e(N_4)]$$

$$+ [(r_{34}L_e + u_{ie})(N_3) - (r_{43}L_i + u_{ei})(N_4)], \tag{A18}$$

and

$$\frac{\mathrm{d}(N_6)}{\mathrm{d}t} = [r_{36}w_e(N_3) - r_{63}L_i(N_6)] + [r_{46}w_e(N_4) - r_{64}L_e(N_6)]$$

$$+ [(r_{16}w_e + s_{ie})(N_1) - (r_{61}w_i + s_{ei})(N_6)].$$

In these expressions, the r_{ij}s represent "reduced" rate constants, determining the transitions shown by broken lines in Fig. 4 *B*. The meaning of subindex "*ij*" is the same as in Appendix 1. The reduced rate constants are expressed in terms of the original rate constants:

$$r_{13} = \frac{r_{w,i}b_{L,i}(b_{w,e}w_e + b_{L,e}L_e + t_{ei})}{D}; \quad r_{31} = \frac{r_{L,i}b_{w,i}(b_{w,e}w_e + b_{L,e}L_e + t_{ei})}{D};$$

$$r_{14} = \frac{r_{w,i}b_{L,e}t_{ie}}{D}; \quad r_{41} = \frac{r_{L,e}b_{w,i}t_{ei}}{D}; \quad r_{16} = \frac{r_{w,i}b_{w,e}t_{ie}}{D}; \quad r_{61} = \frac{r_{w,e}b_{w,i}t_{ei}}{D};$$

$$r_{34} = \frac{r_{L,i}b_{L,e}t_{ie}}{D}; \quad r_{43} = \frac{r_{L,e}b_{L,i}t_{ei}}{D}; \quad r_{36} = \frac{r_{L,i}b_{w,e}t_{ie}}{D}; \quad r_{63} = \frac{r_{w,e}b_{L,i}t_{ei}}{D};$$

$$r_{46} = \frac{r_{L,e}b_{w,e}(b_{w,i}w_i + b_{L,i}L_i + t_{ie})}{D}, \tag{A19}$$

and

$$r_{64} = \frac{r_{w,e}\,b_{L,e}(b_{w,i}\,w_i + b_{L,i}L_i + t_{ie})}{D}.$$

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