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THE EFFECT OF THE CARRIER ASSOCIATION–DISSOCIATION RATE ON MEMBRANE PERMEATION

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SUMMARY

A system of facilitated diffusion is described in which a nonelectrolyte diffuses through the membrane and simultaneously undergoes an association–dissociation reaction with a carrier to form a substrate–carrier complex. The reaction which takes place throughout the membrane facilitates the diffusion. Mathematical expressions for the flow and chemical reaction profiles through the membrane are derived. Criteria are developed to determine whether chemical equilibrium is attained at the membrane surfaces. An expression is derived for the total flow as a function of concentration difference of the permeant across the membrane. The theoretical result is compared with the equations for carrier-mediated transport in which the permeant reacts at the surface only (discontinuous carrier model).

An additional model is introduced in which the carrier is assumed to be an allosteric protein undergoing conformational changes within the membranes. In this model the flow of permeant is found to be an asymmetrical function of concentration differences.

INTRODUCTION

The concept of facilitated transport was introduced by DANIELLI[1] to explain "abnormal" passive permeability of biological membranes. The basic findings, which led to the hypothesis that transport of many substrates is facilitated by interaction with certain membrane components, were reviewed by WILBRANDT AND ROSENBERG[2]. The conventional model for facilitated diffusion assumes that the membrane contains impermeable "carrier" molecules which combine specifically with the permeating substance and aid their passage across the membrane. A number of plausible types of carrier have been discussed[1], *e.g.*, "an expanding and contracting protein", "a rotating macromolecule", "lattice models" and "the diffusing shuttle".

The prevalent view is that the macromolecular carriers can be isolated and identified. Notable steps forward in carrier identification were taken by FOX AND KENNEDY[3] in the isolation of the transfer agent for lactose in the membrane of *Escherichia coli*, by PARDEE[4] in the isolation of a sulfate binding protein in *Salmonella typhimurium*, and by WASSERMAN[5] in the isolation of a transfer agent of calcium

across intestinal epithelium. During recent years, several synthetic models for carrier transport were constructed and studied.[6]

In all the above carrier types, facilitated transport exhibits similar kinetics. However, facilitated transport may be discontinuous, the association–dissociation reaction taking place on the surface only, or continuous, the carrier–permeant complex undergoing an association–dissociation reaction during its passage through the membrane medium. Haemoglobin-mediated transport of oxygen seems to belong to the latter type of facilitated diffusion, and in many synthetic membranes, transport may also be continuous.

This paper delineates in some detail the case of continuous facilitated transport and discusses the effect of the chemical reaction on permeation. A comparison is then made with discrete, or discontinuous, facilitated transport. Finally, transport mediated by an allosteric carrier is considered.

Conditions for continuous, facilitated transport

An important characteristic of facilitated transport is that no discernible chemical change takes place in the medium, whatever the reaction may be within the membrane system. At any point x in the membrane, chemical reaction(s) may be taking place at a rate, J_r. In the case of facilitated transport proceeding in the x direction, across a membrane of thickness Δx, the following requirement must hold:

$$\int_0^{\Delta x} J_r \mathrm{d}x = 0 \qquad (1)$$

The only possible chemical reactions are therefore those of association and dissociation of the transported species with the carrier. If $\int_0^{\Delta x} J_r \, \mathrm{d}x \neq 0$, active transport may take place. This condition exempts facilitated transport from the requirement of asymmetry of an active transport membrane[7], *i.e.*, in active transport there is a net conversion on one side of the membrane of substrates into products.

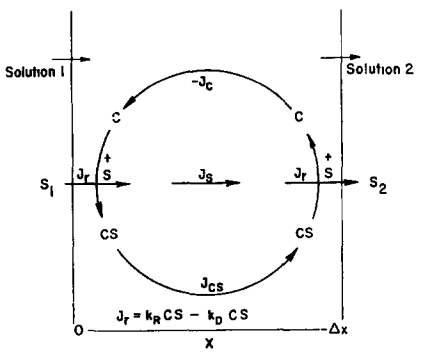


Fig. 1. Continuous carrier model. The permeant (S) enters the membrane from the outer solutions (1) and (2) at points $x = 0$ and $x = \Delta x$. In the membrane phase, the permeant simultaneously diffuses and undergoes an association–dissociation reaction with a carrier (C) to form a substrate–carrier complex (CS). The latter does not pass the barriers at $x = 0$ and $x = \Delta x$. The concentrations of the three components in the membrane phase (S, C and CS), their flows (J_S, J_C and J_{CS}), and the reaction rate (J_r) vary with x. The association (k_R) and dissociation (k_D) rate constants are indicated. The transported species is present in the outer solutions in concentrations S_1 and S_2.

In the case at any point x of the membrane the reaction $C + S \underset{k_D}{\overset{k_R}{\rightleftharpoons}} CS$ takes place, the reaction rate is given by

$$J_r = k_R C \cdot S - k_D CS \tag{2}$$

where C, CS and S are the concentrations of free carrier, of associated carrier and of permeant, respectively, at point x; and k_R and k_D are the rate constants for association and dissociation. The relation between diffusional and chemical flow is given by the equations of continuity:

$$\begin{aligned}
\left(\frac{\partial C}{\partial t}\right)_x &= -\left(\frac{\partial Jc}{\partial x}\right)_t - J_r \\
\left(\frac{\partial S}{\partial t}\right)_x &= -\left(\frac{\partial Js}{\partial x}\right)_t - J_r \\
\left(\frac{\partial CS}{\partial t}\right)_x &= -\left(\frac{\partial Jcs}{\partial x}\right)_t + J_r
\end{aligned} \tag{3}$$

where Jc, Jcs and Js are the flows of the respective components at point x. Under steady-state conditions, all time derivatives vanish, and the partial differentials become total differentials. Hence

$$J_r = -\frac{\mathrm{d}Jc}{\mathrm{d}x} = -\frac{\mathrm{d}Js}{\mathrm{d}x} = \frac{\mathrm{d}Jcs}{\mathrm{d}x} \tag{4}$$

Since the carrier is not permitted to leave or enter the membrane, the flow of carrier in the free and associated condition vanishes on the boundaries

$$J_C^0 = J_C^{\Delta x} = 0, \quad J_{CS}^0 = J_{CS}^{\Delta x} = 0 \tag{5}$$

Combining the second and fourth terms of Eqn. 4,

$$\frac{\mathrm{d}(Jc + Jcs)}{\mathrm{d}x} = 0 \text{ or } Jc + Jcs = \text{constant}$$

In view of Eqn. 5, however, the constant is zero

$$Jc + Jcs = 0 \tag{6}$$

This may be regarded as an expression for the local "circulation" of carrier in a homogeneous medium. A stationary state is maintained by compensatory flow and counterflow at every point of the membrane.

The addition of the third and fourth terms of Eqn. 4 gives

$$\frac{\mathrm{d}(Js + Jcs)}{\mathrm{d}x} = 0 \text{ or } Js + Jcs = \text{constant}$$

The constant is the total flow of transported species and is equal to J_S^t at the boundaries (where Jcs vanishes).

$$Js + Jcs = J_S^0 = J_S^{\Delta x} = J_S^t \tag{7}$$

The flows Js and Jcs are not necessarily constant throughout the membrane, though their sum remains constant.

The dissipation function

The local dissipation function, ϕ, at point x is given by (ref. 8)

$$\phi = Js\left(-\frac{d\mu s}{dx}\right) + Jc\left(-\frac{d\mu c}{dx}\right) + Jcs\left(-\frac{d\mu cs}{dx}\right) + J_r A \tag{8}$$

where the μ's are the chemical potentials of the respective components at point x and the affinity, A, at point x is given by

$$A = \mu c + \mu s - \mu cs \tag{9}$$

with the aid of Eqn. 4 it is seen that

$$J_r A = -\left(\mu c\frac{dJc}{dx} + \mu s\frac{dJcs}{dx} + \mu cs\frac{dJcs}{dx}\right)$$

which upon insertion into Eqn. 8 gives

$$\phi = -\frac{d}{dx}(Js\mu s + Jc\mu c + Jcs\mu cs)^* \tag{10}$$

Integration of Eqn. 8 across the membrane and insertion of the boundary conditions (Eqn. 7) gives

$$\Phi = \int_0^{\Delta x}\phi dx = J_S^t \Delta\mu s \tag{11}$$

where Φ is the total dissipation per unit area and $\Delta\mu_S = \mu_S^0 - \mu_S^{\Delta x}$ is the chemical potential difference of transported species between the two sides of the membrane.

The analysis of the dissipation function shows that although a number of dissipative processes of diffusion and chemical reaction are taking place locally (Eqn. 8), the total dissipation function is composed only of factors which are observed from the outside. The importance of considering the intramembrane circulation lies therefore not in the study of the dissipation function, but rather in the evaluation of the dependence of the flow J_S^t on the driving force $\Delta\mu_S$. Continuous carrier transport imposes a characteristic dependence of flow on force which is considered below.

Flow profiles

We assume that the flow of the components obeys Fick's law locally and that coupling between flows may be neglected.

$$Js = -Ds\frac{dS}{dx};\ Jc = -D\frac{dC}{dx} \text{ and } Jcs = -D\frac{dCS}{dx} \tag{12}$$

where D_S and D are the diffusion constants of permeant and carrier, respectively. For the sake of simplicity we assume that free and associated carrier have the same diffusion constant. Inserting the expressions for Jc and Jcs from Eqn. 12 into Eqn. 6 and integrating, we obtain

$$C + CS = C_t \tag{13}$$

where C_t is the total local carrier concentration, which remains constant irrespective of the variation of C and CS with x.

* Eqn. 10 may be derived from the general consideration of isothermal stationary systems (ref. 9, Chapter III, Eqns. 12 and 20).

Since the exact treatment of Eqn. 12 leads to nonhomogeneous nonlinear differential equations, whose solution cannot be expressed in manageable analytical form, we shall consider in the following only the case of a system close to equilibrium. In this case the ratio of the concentration difference of permeant and its equilibrium concentration is very small ($\Delta S/S \ll 1$); the local concentration shows a slight deviation from equilibrium, *i.e.*,

$$\delta S = S - \bar{S},\ \delta C = C - \bar{C},\ \delta CS = CS - \overline{CS} \tag{14}$$

where $\bar{C}$ and $\overline{CS}$ are equilibrium concentrations of free and associated carrier, and $\bar{S}$ is the equilibrium concentration of the permeant. The deviations are dependent on x. Substituting S, C, and CS from Eqn. 14 into Eqn. 2

$$J_r = k_R(\bar{C} + \delta C)(\bar{S} + \delta S) - k_D(\overline{CS} + \delta CS)$$

or

$$J_r = k_R\bar{C}\delta S + k_R\bar{S}\delta C - k_D\delta CS \tag{15}$$

In Eqn. 15 the equilibrium condition $k_R\bar{C}\cdot\bar{S} = k_D\overline{CS}$ has been introduced, and the nonlinear term ($k_R\delta C\delta S$) has been neglected.

Substituting Eqn. 12 for the flows of the components into Eqn. 4, and with the assumption that the diffusion constants are independent of x,

$$J_r = D_S\frac{d^2S}{dx^2} = D\frac{d^2C}{dx^2} = -D\frac{d^2CS}{dx^2} \tag{16}$$

Differentiating J_r in Eqn. 15 twice and substituting for the second differentials of the concentrations, according to Eqn. 16, we obtain

$$\frac{d^2J_r}{dx^2} = \Lambda^{-2}J_r \tag{17}$$

where

$$\Lambda^{-2} = \frac{k_R\bar{S} + k_D}{D} + \frac{k_R\bar{C}}{D_S} \tag{18}$$

The parameter Λ has been introduced in a general way by FRIEDLANDER AND KELLER[10]. It has the dimensions of a distance and was termed by the authors the "relaxation distance". When there is a flow of one of the reactants across a boundary of a reacting system, the deviation from chemical equilibrium is greatest at the boundary. The deviation decreases with distance from the interface and becomes negligible at points further away than the relaxation distance. The ratio of Λ to the thickness of the diffusion path (*e.g.*, membrane, film or boundary layer thickness) expresses the effect of the chemical reaction on the diffusion flow and determines the type of kinetics to be applied.

Eqn. 17 may be solved to give J_r as a function of the distance x from the surface. Inserting the boundary condition (Eqn. 1, Eqn. 5, and Eqn. 7) the following expression is obtained (Appendix: Eqn. I–4).

$$J_r = J_r^0\left[\cosh\left(\frac{x}{\Lambda}\right) - \operatorname{cotgh}\left(\frac{\Delta x}{2\Lambda}\right)\sinh\frac{x}{\Lambda}\right] \tag{19}$$

where J_r^0 is the rate of reaction at $x = 0$. It will be noted that at $x = \Delta x$

$$J_r^{\Delta x} = -J_r^0 \text{ and at } x = \frac{\Delta x}{2},\ J_r^{\Delta x/2} = 0$$

So that for the quasiequilibrium case, assumed in this treatment, the reaction rates are symmetrical around the center of the membrane—where the reaction is at equilibrium. The absolute value of J_r is always less than that of J_r^0. Fig. 2 represents the ratio of J_r to J_r^0 as a function of x for two values of $\Delta x/2\Lambda$.

An important question is which part of the substrate passes while bound to the carrier and which permeates in a free state. The flow of the carrier–substrate complex J_{CS} is given by Eqn. I–5, which in combination with Eqn. I–9 of Appendix I gives

$$J_{CS} = \alpha J_S^t \left(\operatorname{tgh} \frac{\Delta x}{2\Lambda} \sinh \frac{x}{\Lambda} - \cosh \frac{x}{\Lambda} + 1 \right) \tag{20}$$

where

$$\alpha = \frac{k_R \bar{C} \Lambda^2}{D_S}$$

or upon inserting the value of Λ^2 from Eqn. 18

$$\alpha = \frac{1}{1 + \dfrac{D_S}{D} \dfrac{(k_R \bar{S} + k_D)}{k_R \bar{C}}} \tag{21}$$

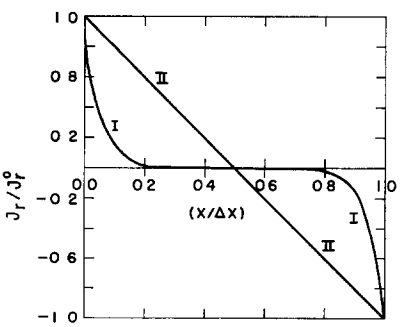
From the equilibrium conditions $k_R \bar{C} \cdot \bar{S} = k_D \overline{CS}$ and Eqn. 13 ($\bar{C} + \overline{CS} = C_t$)

$$\bar{C} = \frac{K_S C_t}{K_S + \bar{S}} \tag{22}$$

where $K_S = k_D/k_R$ is the dissociation constant of the carrier–substrate complex.

Inserting Eqn. 22 into Eqn. 21 and rearranging

$$\alpha = \frac{1}{1 + \dfrac{D_S K_S}{D C_t} \left(1 + \dfrac{\bar{S}}{K_S} \right)^2} \tag{23}$$



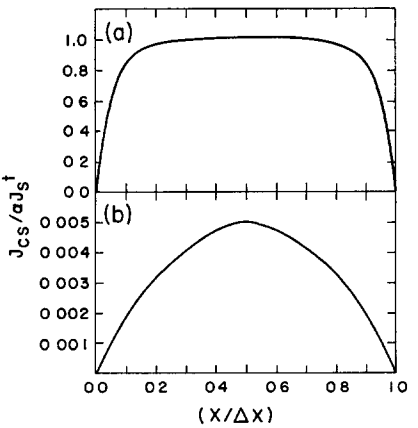


Fig. 2. The dependence of J_r on x for $\Delta x/2\Lambda = 10$ (curve I) and $\Delta x/2\Lambda = 0.1$ (curve II). J_r^0 is the reaction velocity at the surface $x = 0$ (*cf.* Eqn. 20).

Fig. 3. The dependence of J_{CS} on x for $\Delta x/2\Lambda = 10$ (a) and $\Delta x/2\Lambda = 0.1$ (b). J_S^t is the total flow of substrate, for the interpretation of $\alpha = k_R \bar{C} \Lambda^2/D_S$, *cf.* text.

For a medium in which the free substrate diffuses more slowly than the carrier ($D_S/D < 1$), or a medium rich in carrier and with low dissociation constant ($C_t > K_S$), α is close to unity and carrier transport is preferred. On the other hand, during rapid free diffusion and high substrate concentration as compared with the dissociation constant, *i.e.*, $\overline{S}/K_S \gg 1$, α goes to zero so that the contribution of J_{CS} to the total flow vanishes.

Fig. 3 represents $J_{CS}/\alpha J_S^t$ for a given value of α and at two values of $\Delta x/2\Lambda$. It will be observed that for large $\Delta x/2\Lambda$, *e.g.* $\Delta x/2\Lambda = 10$, carrier-mediated transport constitutes a large fraction of the total transport. It may be readily shown that in this case $J_S^t/J_{CS} \cong \alpha$ in the major part of the membrane, except in the neighbourhood of the surfaces where J_{CS} goes to zero.

On the other hand for small $\Delta x/2\Lambda$, *e.g.* $\Delta x/2\Lambda = 0.1$, J_{CS}/J_S^t is a small fraction of α and even at $x = \Delta x/2\Lambda$, $J_{CS}/J_S^t \to \alpha/2(\Delta x/2\Lambda)^2$, which is only 0.5 % of the maximal value for J_{CS}/J_S^t for large $\Delta x/2\Lambda$.

The total flow

Since the total permeant flow across the membrane is given by $J_S^t = J_S + J_{CS}$ (Eqn. 7), we obtain upon inserting J_S and J_{CS} from Eqn. 12 and integrating over x, from 0 to Δx:

$$J_S^t = D_S\frac{\Delta S}{\Delta x} + D\frac{\Delta CS}{\Delta x} \tag{24}$$

where $\Delta S = S^0 - S^{\Delta x}$ and $\Delta CS = CS^0 - CS^{\Delta x}$.

The total flow of S is therefore represented additively by a term giving the free diffusion of transported species and a term for facilitated diffusion. If the concentrations of the solutions on both sides of the membrane are known, the concentration of S on the membrane boundaries may be calculated, provided the distribution coefficient (K_n) between solution and membrane medium is also known. The evaluation of ΔCS, however, requires a closer analysis.

By Eqn. 12 ΔCS is related to J_{CS} through the expression

$$\Delta CS = \frac{1}{D}\int_0^{\Delta x} J_{CS}\mathrm{d}x$$

Upon inserting the value of J_{CS} and integrating (*cf.* Appendix I) a relation between ΔCS and J_r^0 is obtained

$$\Delta CS = \frac{2\Lambda^2\chi}{D}J_r^0 \tag{25}$$

where

$$\chi = \frac{\Delta x}{2\Lambda}\operatorname{cotgh}\frac{\Delta x}{2\Lambda} - 1 \tag{26}$$

Another relation between ΔCS and J_r may be obtained as follows: as shown above at $\Delta x/2$, $J_r = 0$, so that C, CS and S assume equilibrium values at this point, or:

$$\bar{S} = S^{\Delta x/2},\ \bar{C} = C^{\Delta x/2} \text{ and } \overline{CS} = CS^{\Delta x/2} \tag{27}$$

Again making use of Eqn. 12 we may write

$$CS^{\Delta x/2} = CS^0 - \frac{1}{D}\int_0^{\Delta x/2} J_{CS}\,dx$$
$$CS^{\Delta x} = CS^{\Delta x/2} - \frac{1}{D}\int_{\Delta x/2}^{\Delta x} J_{CS}\,dx \tag{28}$$

An inspection of Eqn. 20 shows that J_{CS} is symmetrical around its maximal value at $\Delta x/2$. Hence the integral of J_{CS} from 0 to $\Delta x/2$ is equal to its integral from $\Delta x/2$ to Δx. Upon subtracting the expressions in Eqn. 28 and rearranging we obtain therefore

$$CS^{\Delta x/2} = \frac{CS^0 + CS^{\Delta x}}{2}$$

or with Eqn. 27

$$\overline{CS} = \frac{CS^0 + CS^{\Delta x}}{2} \tag{29}$$

In a similar way it may be shown that

$$\overline{C} = \frac{C^0 + C^{\Delta x}}{2} \text{ and } \bar{S} = \frac{S^0 + S^{\Delta x}}{2} \tag{30}$$

hence

$$S^0 = \bar{S} + \frac{\Delta S}{2};\quad C^0 = \bar{C} + \frac{\Delta C}{2} \text{ and } CS^0 = \overline{CS} + \frac{\Delta CS}{2} \tag{31}$$

The reaction rate on the surface $x = 0$ is

$$J_r^0 = k_R C^0 S^0 - k_D CS^0$$

which upon insertion of Eqn. 31 and cancelling the equilibrium term $K_S\overline{CS} = \overline{C}\cdot\overline{S}$, gives

$$J_r^0 = \frac{k_R}{2}\,[\bar{C}\Delta S - (K_S + \bar{S})\Delta CS] \tag{32}$$

Inserting J_r^0 from Eqn. 32 into Eqn. 25, we obtain the required expression for ΔCS

$$\Delta CS = \frac{\bar{C}\Delta S}{(K_S + \bar{S}) + (D/k_R\chi\Lambda^2)} \tag{33}$$

The value $\overline{C}$ is given by Eqn. 22, while Λ^2 is defined by Eqn. 18. Inserting both expressions we finally obtain

$$\Delta CS = \frac{K_S C_t \Delta S}{(1 + 1/\chi)(K_S + \bar{S})\left(K_S + \bar{S} + \dfrac{DK_S C_t}{D_S(K_S + \bar{S})(1 + \chi)}\right)} \tag{34}$$

Eqn. 34 may now be used to evaluate the total flow according to Eqn. 24

$$J_S^t = D_S \frac{\Delta S}{\Delta x} + \frac{DK_S C_t}{(1 + 1/\chi)(K_S + \bar{S})\left[K_S + \bar{S} + \frac{DK_S C_t}{D_S(K_S + \bar{S})(1 + \chi)}\right]} \frac{\Delta S}{\Delta x} \tag{35}$$

In the case where $\Delta x/2\Lambda \gg 1$, $\chi \to (\Delta x/2\Lambda - 1) \gg 1$, Eqn. 35 reduces to

$$(J_S^t)_{\Delta x/2\Lambda \gg 1} = D_S \frac{\Delta S}{\Delta x} + \frac{DK_S C_t}{(K_S + \bar{S})^2} \frac{\Delta S}{\Delta x} \tag{36}$$

Eqn. 36 is the expression for facilitated transport with local chemical equilibrium. This statement corresponds to the conclusion of FRIEDLANDER AND KELLER[10] that local equilibrium may be assumed if the thickness of the total diffusion path (Δx) is appreciably larger than the relaxation distance (Λ). On the other hand if $\Delta x/2\Lambda \ll 1$, $x \to 1/3\,(\Delta x/2\Lambda) \ll 1$ and $1/\chi \gg 1$ so that the term for facilitated transport goes to

$$\frac{DK_S C_t}{\left[(K_S + \bar{S})^2 + \frac{DK_S C_t}{D_S(K_S + \bar{S})}\right]} \frac{\Delta S}{\Delta x}$$

which for very small values of χ vanishes in comparison with the terms for non-facilitated transport $D_S \Delta S/\Delta x$.

Comparison with discontinuous carrier transport

The only carrier kinetics treated extensively in the literature is that of a discontinuous model, in which permeant is assumed to interact with the carrier solely at the membrane surfaces[2]. It is generally postulated that the transport process is diffusion-controlled so that surface equilibrium may be assumed.

If chemical surface equilibrium is not assumed, facilitated transport is given by Eqn. 37

$$J_S = \frac{PK_S C_t}{(K_S + S_1)(K_S + S_2) + \frac{2P}{k_D \Delta x} K_S(K_S + \bar{S})} \Delta S \tag{37}$$

where S_1 and S_2 are the permeant concentrations on both sides of the membrane and the permeability coefficient P is related to the diffusion coefficient D by

$$P = \frac{D}{\Delta x} \tag{38}$$

For the case of surface equilibrium Eqn. 37 reduces to the conventional expression

$$J_S^e = \frac{PK_S C_t}{(K_S + S_1)(K_S + S_2)} \Delta S \tag{39}$$

If $S_1 \cong S_2 \cong \bar{S}$ Eqns. 37 and 39 may be written as

$$J_S = \frac{PK_S C_t \Delta S}{(K_S + \bar{S})^2}\left[1 + \frac{2PK_S}{k_D \Delta x (K_S + \bar{S})}\right]^{-1} = J_S^e\left[1 + \frac{2P}{\Delta x(k_R \bar{S} + k_D)}\right]^{-1} \tag{40}$$

According to Einstein, the relaxation time (τ_{diff}) of a diffusional process is related to the path Δx by the well-known equation $2D\tau_{diff} = (\Delta x)^2$. But since

$$\frac{2P}{\Delta x} = \frac{2D}{(\Delta x)^2}, \quad \frac{2P}{\Delta x} = \frac{1}{\tau_{diff}} \tag{41}$$

To estimate the relaxation time of the chemical reaction, we consider Eqn. 15 for $\delta S = 0$, *i.e.*, for the case in which the S of the outer compartments is kept "buffered". In this case, $\delta C = -\delta CS$ and the rate of chemical reaction is given by a perturbation of δCS (ref. 11), *i.e.*,

$$\frac{d\delta CS}{dt} = -(k_R\bar{S} + k_D)\delta CS \tag{42}$$

or $d\delta CS/dt = -1/\tau_{chem}\delta CS$, where

$$1/\tau_{chem} = k_R\bar{S} + k_D \tag{43}$$

Inserting Eqn. 41 and Eqn. 43 into Eqn. 40 we find that

$$J_S = J_S^e\left(1 + \frac{\tau_{chem}}{\tau_{diff}}\right)^{-1} \tag{44}$$

When the chemical relaxation time is much shorter than the diffusional time $J_S = J_S^e$ as expected.

If we assume that the carrier is a macromolecule to which the permeant is bound, τ_{chem} is the adsorption–desorption relaxation time and τ_{diff} is the relaxation time for its conformational transition. KIRSCHNER *et al.*[12] have measured relaxation times for the binding of nicotinamide–adenine dinucleotide to yeast D-glyceraldehyde 3-phosphate and the conformational transition of the enzyme. These authors find for this system that $\tau_{chem} = 1.4\cdot10^{-4}$—$1.4\cdot10^{-3}$ sec and $\tau_{diff} = 5$ sec.

In this case the statement $J_S = J_S^e$ and the generally applied assumption of local equilibrium at the surface are justified.

An allosteric model for carrier transport

The expanding and contracting carrier assumed above may be considered an

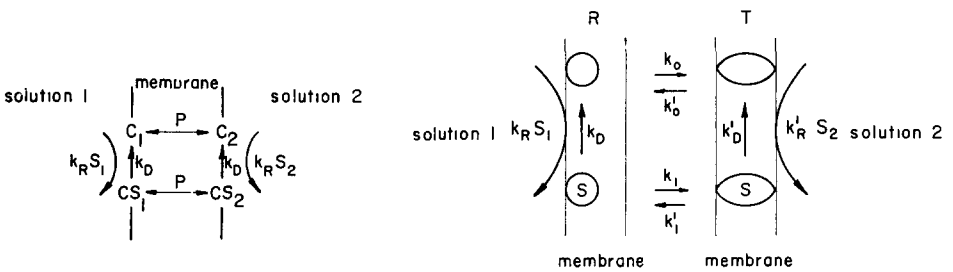


Fig. 4. Discrete carrier model. The permeant undergoes an association–dissociation reaction of the membrane surfaces with the carrier (C) to form the carrier–substrate complex (CS). The latter does not leave the membrane phase. The permeation constants (P) for the free and associated carrier are equal. The association (k_R) and dissociation (k_D) rate constants are equal on both sides. The concentrations of transported species in solutions 1 and 2 are S_1 and S_2.

Fig. 5. Allosteric carrier model. The protein may assume the two configurations R and T within the membrane phase; the rates of conformational change are k_0 and k'_0 for the unassociated and k_1 and k'_1 for the associated protein. The transported species only binds to the protein in the R form on side 1 of the membrane (association and dissociation rate constants k_R and k_D) and to the protein T form on side 2 (association and dissociation rate constants k'_R and k'_D). The transported species is present in solutions 1 and 2 at concentrations S_1 and S_2.

allosteric protein.[13] The allosteric model will be used in its simplest form: (*i*) each subunit has one binding site for the transported species; it acts independently and there are no cooperative transitions; (*ii*) the effect of regulatory sites for inhibitors or activators will not be considered. The only assumptions of the model for allosteric interactions considered in the conclusions concerning the flow pattern are: (*a*) the conformational transition of the protein; (*b*) the existence of a different binding affinity of the permeant on each side of the membrane for the two conformations of the carrier.

The two forms of the protein, R and T (Fig. 5), have different dissociation constants for the transported species: $K_S = k_D/k_R$ and $K'_S = k'_D/k'_R$, respectively. The equilibrium constants for conformational transitions of $R \rightleftharpoons T$ in free and associated protein are, respectively, $L_0 = k_0/k'_0$ and $L_1 = k_1/k'_1$. The constants are related to each other by the principle of microscopic reversibility

$$L_1 = (K_S/K'_S)L_0 \tag{45}$$

KIRSCHNER *et al.*[12] determined the kinetics of allosteric transitions, while HILL AND KEDEM[14] have treated the steady-state kinetics of a similar membrane-flow system. The expression for total flow is (*cf.* Appendix II):

$$J_S = \frac{k_0 k'_1 K_S C_t \Delta S}{(S_1 + K_S)(k'_1 S_2 + k'_0 K'_S) + (S_2 + K'_S)(k_1 S_1 + k_0 K_S)} \tag{46}$$

In Fig. 6, J_S *versus* ΔS is represented for the conventional case ($K_S = K'_S, L_1 = L_0 = 1$) as well as for the allosteric case. The striking feature in even the simplest assumption of an allosteric model is the asymmetry of flow as a function of concentration difference, for example, if $S_2 = 0$ or $S_1 = 0$, $J_S/k'_1 C_t$ assumes the values 0.083 and —0.048, respectively.

This model may be extended to include the possibility that an assembly of carrier proteins ("subunits") undergoes cooperative transitions. Changes in asymmetric permeabilities, for example those that occur in nerves, may be brought about by cooperative transitions of the total membrane lattice.

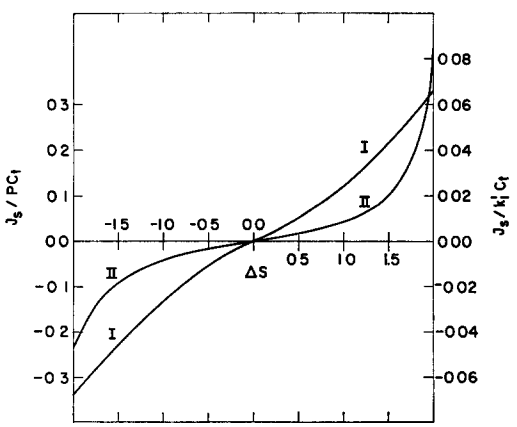


Fig. 6. Dependence of flow of transport species (J_S) on concentration difference (ΔS) between the two sides of the membrane, for conventional carrier transport (Curve I, left scale) and for the allosteric model (Curve II, right scale). In both cases a constant average concentration is taken of $S = 1$ mole/l. Curve I is taken from Eqn. 37 for $K_S = 1$ mole/l. In Curve II (Eqn. 46), $K_S = 1$ mole/l, $K'_S = 0.1$ mole/l, $L_0 = 1$ and $L_1 = 10$ and $k_0 = k'_0 = k'_1$.

APPENDIX I

The general solution of Eqn. 17 is

$$J_r = a_1 e^{x/\Lambda} + a_2 e^{-x/\Lambda} \tag{I-1}$$

where a_1 and a_2 are integration constants. At $x = 0$

$$J_r^0 = a_1 + a_2 \tag{I-2}$$

From $\int_0^{\Delta x} J_r dx = 0$ (*cf.* Eqn. 1) we obtain

$$a_2 - a_1 = J_r^0 \operatorname{cotgh}(\Delta x/2\Lambda) \tag{I-3}$$

Insertion of Eqn. I-3 and Eqn. I-2 into Eqn. I-1 gives

$$J_r = J_r^0 [\cosh(x/\Lambda) - \operatorname{cotgh}(\Delta x/2\Lambda) \sinh(x/\Lambda)] \tag{I-4}$$

From $J_{CS} = \int_0^x J_r dx$ (*cf.* Eqn. 4) we obtain

$$J_{CS} = J_r^0 \{\sinh(x/\Lambda) - \operatorname{cotgh}(\Delta x/2\Lambda)(\cosh(x/\Lambda) - 1)\} \tag{I-5}$$

In order to express J_{CS} in terms of total flow we differentiate Eqn. 15:

$$\frac{\partial J_r}{\partial x} = k_R \bar{C} \frac{dS}{dx} + k_R \bar{S} \frac{dC}{dx} - k_D \frac{dCS}{dx} \tag{I-6}$$

At $x = 0$: $(dC/dx)_0 = -(1/D) J_C^0 = 0$, $(dCS/dx)_0 = -(1/D) J_{CS}^0 = 0$ and $(dS/dx)_0 = -(1/D_S) J_S^t$ (*cf.* Eqns. 5, 7 and 12). So that

$$\left(\frac{\partial J_r}{\partial x}\right)_0 = -\frac{k_R}{D_S} \bar{C} J_S^t \tag{I-7}$$

From Eqn. (I-4), however, at $x = 0$ we obtain

$$\left(\frac{\partial J_r}{\partial x}\right)_0 = -\frac{J_r^0}{\Lambda} \operatorname{cotgh}(\Delta x/2\Lambda) \tag{I-8}$$

Combining (I-7) and (I-8)

$$J_r^0 = \frac{k_R}{D_S} \bar{C} \Lambda (\operatorname{tgh}(\Delta x/2\Lambda)) J_S^t \tag{I-9}$$

Insertion of Eqn. I-9 into Eqn. I-5 gives Eqn. 21.

From $CS^x = CS^0 - (1/D) \int_0^x J_{CS} dx$ (*cf.* Eqn. 12) we obtain, by integrating Eqn. I-5

$$CS^x = CS^0 - \frac{\Lambda^2 J_r^0}{D} [\cosh(x/\Lambda) - 1 + \operatorname{cotgh}(\Delta x/2\Lambda)((x/\Lambda) - \sinh(x/\Lambda))] \tag{I-10}$$

Inserting $x = \Delta x$ into Eqn. I-10 gives

$$J_r^0 = (D/2\Lambda^2)[(\Delta x/2\Lambda) \operatorname{cotgh}(\Delta x/2\Lambda) - 1]^{-1} \Delta CS \tag{I-11}$$

APPENDIX II

The assumption of local equilibrium of the surface reaction yields:

$$\begin{aligned} R \cdot S_1 &= K_S R S \\ T \cdot S_2 &= K'_S T S \end{aligned} \tag{II-1}$$

where R, T, RS and TS are the concentrations of the protein in the four states. The "circulation equation" (Eqn. 6) and "total carrier equation" (Eqn. 13) hold for this model:

$$k_0R - k'_0T + k_1RS - k'_1TS = 0 \tag{II-2}$$

$$R + RS + T + TS = C_t \tag{II-3}$$

Substituting for R and T according to Eqn. II-1

$$\begin{aligned} &RS\left(k_0\frac{K_S}{S_1} + k_1\right) - TS\left(\frac{k'_0K'_S}{S_2} + k'_1\right) = 0 \\ &RS(1 + K_S/S_1) + TS(1 + K'_S/S_2) = C_t \end{aligned} \tag{II-4}$$

solving Eqn. II-4 for RS and TS

$$RS = \frac{(k'_0K'_S/S_2 + k'_1)C_t}{(k_0K_S/S_1 + k_1)(1 + K'_S/S_2) + (k'_0K'_S/S_2 + k'_1)(1 + K_S/S_1)} \tag{II-5}$$

$$TS = \frac{(k_0K_S/S_1 + k_1)C_t}{(k_0K_S/S_1 + k_1)(1 + K'_S/S_2) + (k'_0K'_S/S_2 + k'_1)(1 + K_S/S_1)} \tag{II-6}$$

The rate of transport is given by

$$J_S = k_1RS - k'_1TS \tag{II-7}$$

Introducing Eqn. II-5 and Eqn. II-6 into Eqn. II-7 gives

$$J_S = \frac{k_1k'_0K'_S/S_2 - k'_1k_0K_S/S_1}{(k_0K_S/S_1 + k_1)(1 + K'_S/S_2) + (k'_0K'_S/S_2 + k'_1)(1 + K_S/S_1)} \tag{II-8}$$

The microscopic reversibility condition (Eqn. 45) requires

$$K_S/K'_S = L_1L_0 = k_1k'_0/k'_1k_0 \text{ or } k_1k'_0K'_S = k'_1k_0K_S \tag{II-9}$$

Introducing Eqn. II-9 into Eqn. II-8 and multiplying numerator and denominator by S_1S_2 gives Eqn. 46.

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