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COUPLING IN SECONDARY TRANSPORT

EFFECT OF ELECTRICAL POTENTIALS ON THE KINETICS OF ION LINKED CO-TRANSPORT

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SUMMARY

In a previous paper kinetic equations of secondary active transport by co-transport have been derived. In the present paper these equations have been expanded by including the effect of an electrical potential difference in order to make them applicable to the more realistic systems of secondary active transport driven by the gradients of Na^+ or H^+. Thermodynamically an electrical potential difference is as a driving force fully exchangeable with an equivalent chemical potential difference. This is not necessarily so for the kinetics of co-transport. It is not always the same whether a given difference in electrochemical activity of the driver ion is mainly osmotic, i.e. due to difference in concentration, or electric, i.e. due to a difference in the electrochemical activity coefficient. In most cases a difference in concentration is more effective in driving co-transport than is an equivalent difference in electrical potential leading to the same difference in electrical activity. The effectiveness of the latter highly depends on the model, whether it is of the affinity type or of the velocity type, but also on whether the loaded or the unloaded carrier bears an electrical charge. With the same electrical potential difference co-transport is as a rule faster if the ternary complex rather than the empty carrier is charged. Also the "standard parameters", (see Glossary, page 62) J_{max} and K_m, of the overall transport respond differently to the introduction of an electrical potential difference, depending on the model. So an electrical potential difference will mostly affect K_m if the loaded carrier is ionic, and mostly J_{max} if the empty carrier is ionic, provided that the mobility of the loaded carrier is greater than that of the empty one. On the other hand, distinctive criteria between affinity type and velocity type models are partly affected by an electrical potential difference. If the translocation steps of loaded and unloaded carrier are no longer rate limiting for the overall transport, electrical effects on the transport rate are bound to vanish as does the activation by co-transport.

INTRODUCTION

It is now widely accepted that the active transport of several solutes, especially amino acids and sugars, is driven by the electrochemical potential gradient of an electrolyte ion [1, 2]. This assumption, known as the "gradient hypothesis", implies that the two fluxes concerned, that of the nonelectrolyte and that of the ion, are energetically coupled to each other by "co-transport", presumably through the formation of a "ternary complex" between carrier, substrate and co-ion at a fixed stoichiometry. The "gradient hypothesis" appears to apply to animal cells and microorganisms alike, the Na^+ predominating as co-ion in the former, and H^+, in the latter kind of cell.

In a previous paper the kinetics of co-transport, based on the assumption of a ternary complex, has been analysed mathematically. First a generalized equation was developed in which all possible interactions between the carrier and its ligands were incorporated [3]. From this equation several "reduced models" were derived on the basis of the assumption that binding of the co-substrate to the carrier promotes the active transport of the main substrate by one or a combination of two distinct effects. An increase of the affinity of the carrier for the main substrate (affinity type) and/or an increase of the penetration rate of the complex (velocity type). It could be shown that the "pure" types in which either the one or the other effect were assumed to predominate, should exhibit characteristic differences in their kinetics, notably in their "standard parameters": maximum velocity (see Glossary, p. 62) (J_{max}) and Michaelis constant (K_m), and in their dependence on certain experimental conditions.

Even though in most cases of co-transport the "driving" solute is an ion (Na^+ or H^+) whose electrochemical potential gradient should strongly depend also on an electrical potential difference across the transporting membrane, the electrical potential was not explicitly considered in the above nor in other, similar treatments [1, 4, 5]. Hence the above equation would strictly apply to the "short circuit" condition only. Since this condition can hardly be verified in many systems, experimentally, it is highly desirable to overcome this shortcoming by properly introducing electrical effects.

From the energetic point of view, the implementation of an electrical potential in the above equations poses no special problem. Any contribution of the electrical potential difference to the driving force is usually taken care of by adding an electrical potential term to the chemical potential difference of an ion, thus arriving at the electrochemical potential difference of the ion. Analogously, one might try to adapt the kinetic equations of co-transport to electrical forces by inserting the electrochemical activities of the ions rather than their chemical activities or concentrations. In other words, each ion concentration or activity should be multiplied by the appropriate electrochemical activity coefficient, ξ^+. For various reasons, however, this procedure raises some problems. Firstly, the permeability coefficient (p) of each charged particle is itself a function of the electrical field, even for homogenious membrane phase. It will be shown below, however, that in the presence of a high nonelectric barrier which has to be overcome by all particles penetrating the membrane, such a function may be neglected. In addition, an electric field may induce structural alterations of membrane components involved in transport, which may also effect the permeability coefficients of ions, or even those of nonelectrolytes, and may be sometimes associated with rectification phenomena. In the present model, however, such special effects will not be considered, but one should be aware that they may be present. On the other hand, not all reaction rates, crucially involving a charged particle, depend on an electrical field. For instance, binding and release of an ion by a carrier should not depend on it, since the electrical activity coefficient would be cancelled.

Hence, the formal procedure can be simplified by omitting electrical potential terms from all dissociation constants, so that in the end only the rate coefficients (p_i) of the charged particles across the membrane would contain an electrical potential term. Which of the various carrier species involved in the transport is charged, depends on the charge of the carrier molecule itself and on the stoichiometry of the ion binding. In the following treatment, the two most simple possibilities in this respect are taken into account, namely that either the empty carrier or the ternary complex is neutral. In order to facilitate practical application of these derivations to actual systems, the main emphasis is put on the "reduced models", analogous to those presented in the previous paper [3]. It will be shown that by introducing the various effects of the electrical potential difference, the kinetic characteristics of the previously developed models are modified in various ways depending on which of the mobile carrier species are charged, and which of the steps involved limit the overall transport rate.

In the beginning, we shall present a more general equation, analogous to that given in the previous paper, but introducing special terms and factors representing the effects of electrical potentials. In order to keep the derivation simple and lucid, we make the following assumptions, similar to those made in our previous treatment.

1. Only two solute species are transported, the main transportee (a nonelectrolyte) (A) and its co-ion (B). Each binds to its appropriate site of a mobile carrier (X), at the stoichiometric ratio 1 : 1.

2. The overall transport rate is determined by the translocation step. This assumption implies that the reaction between carrier and solutes in the boundaries is very rapid and frequent as compared to the translocation step, so that quasi equilibrium between free and bound ligands can be assumed all the time.

GENERAL MODEL

The carrier X transportee A and effector B may have the charges z, z_a and z_b, respectively. The electrical potential at the two sides of the membrane ′ and ″ may be $-(\psi/2)$ and $+(\psi/2)$, respectively. The equilibrium between carrier and ligands at the phase boundaries is determined by the following equations.

$$\frac{x'\cdot a'}{ax'} = K_a' \qquad \frac{x'\cdot b'}{bx'} = K_b' \qquad \frac{x'\cdot a'\cdot b'}{abx'} = K_a'\cdot K_b'/r' \tag{1}$$

and analogues for ″ side.
The following relationships hold since the total amount of carrier is constant:

$$x'+ax'+bx'+abx'+x''+bx''+abx'' = x_T \tag{2}$$

Since the carrier system is in the steady state

$$\begin{gathered}\tilde{p}_o'\cdot x'+\tilde{p}_a'\cdot ax'+\tilde{p}_b'\cdot bx'+\tilde{p}_{ab}'\cdot abx' = \\ \tilde{p}_o''\cdot x''+\tilde{p}_a''\cdot ax''+\tilde{p}_b''\cdot bx''+\tilde{p}_{ab}''\cdot abx''\end{gathered} \tag{3}$$

The dependence of the permeation probabilities on the electrical potential difference is described as follows:

$$\tilde{p}_o' = p_o' \cdot \phi_o(\psi) \cdot \xi^{z/2}$$
$$\tilde{p}_a' = p_a' \cdot \phi_a(\psi) \cdot \xi^{(z+z_a)/2}$$
$$\tilde{p}_b' = p_b' \cdot \phi_b(\psi) \cdot \xi^{(z+z_b)/2}$$
$$\tilde{p}_{ab}' = p_{ab}' \cdot \phi_{ab}(\psi) \cdot \xi^{(z+z_a+z_b)/2}$$
$$\tilde{p}_o'' = p_o'' \cdot \phi_o(\psi) \cdot \xi^{-z/2}$$
$$\tilde{p}_a'' = p_a'' \cdot \phi_a(\psi) \cdot \xi^{-(z+z_a)/2}$$
$$\tilde{p}_b'' = p_b'' \cdot \phi_b(\psi) \cdot \xi^{-(z+z_b)/2}$$
$$\tilde{p}_{ab}'' = p_{ab}'' \cdot \phi_{ab}(\psi) \cdot \xi^{-(z+z_a+z_b)/2}$$
$$\xi = \exp\left[-F\psi/RT\right] \tag{4}$$

The p are the permeation probabilities at $\psi = 0$. The $\phi(\psi)$ are functions of the potential difference depending on the transfer mechanism and describing potential difference induced alterations of the membrane structure. For a simple Eyring' mechanism the $\phi(\psi)$ are 1. From the second law of thermodynamics follows for equilibrium.

$$\frac{a''}{a'_{\text{equ.}}} = \xi^{z_a} \qquad \frac{b''}{b'_{\text{equ.}}} = \xi^{z_b} \qquad \frac{x''}{x'_{\text{equ.}}} = \frac{\tilde{p}_o'}{\tilde{p}_o''} \qquad \frac{ax''}{ax'_{\text{equ.}}} = \frac{\tilde{p}_a'}{\tilde{p}_a''} \qquad \text{etc.} \tag{5}$$

Inserting Eqn. 5 into Eqn. 1 one arrives at the following side independent constants.

$$K_a' \cdot \frac{p_o' \cdot \phi_o(\psi)}{p_a'' \cdot \phi_a(\psi)} = K_a'' \cdot \frac{p_o'' \cdot \phi_o(\psi)}{p_a'' \cdot \phi_a(\psi)} = \bar{K}_a$$
$$K_b' \cdot \frac{p_o' \cdot \phi_o(\psi)}{p_b' \cdot \phi_b(\psi)} = K_b'' \cdot \frac{p_o'' \cdot \phi_o(\psi)}{p_b'' \cdot \phi_b(\psi)} = \bar{K}_b$$
$$r' \frac{p_o' \cdot p_{ab}' \cdot \phi_o(\psi) \cdot \phi_{ab}(\psi)}{p_a' \cdot p_b' \cdot \phi_a(\psi) \cdot \phi_b(\psi)} = r'' \frac{p_o'' \cdot p_{ab} \cdot \phi_o(\psi) \cdot \phi_{ab}(\psi)}{p_a'' \cdot p_b'' \cdot \phi_a(\psi) \cdot \phi_b(\psi)} = \bar{r} \tag{6}$$

The unidirectional fluxes are

$$J_a' = \tilde{p}_a' \cdot ax' + \tilde{p}_{ab}' \cdot abx' = \bar{\alpha}'(1+\bar{r}\bar{\beta}')V''/R$$
$$J_a'' = \tilde{p}_a'' \cdot ax'' + \tilde{p}_{ab}'' \cdot abx'' = \bar{\alpha}''(1+\bar{\beta}'')V'/R$$
$$J_b' = \tilde{p}_b' \cdot bx' + \tilde{p}_{ab}' \cdot abx' = \bar{\beta}'(1+\bar{r}\bar{\alpha}')V''/R$$
$$J_b'' = \tilde{p}_b'' \cdot bx'' + \tilde{p}_{ab}'' \cdot abx'' = \bar{\beta}''(1+\bar{r}\bar{\alpha}'')V'/R$$

with

$$\bar{\alpha}' = \frac{a'}{\bar{K}_a} \cdot \xi^{z_a/2} \qquad \bar{\alpha}'' = \frac{a''}{\bar{K}_a} \cdot \xi^{-z_a/2} \qquad \bar{\beta}' = \frac{b'}{\bar{K}_b} \cdot \xi^{z_b/2} \qquad \bar{\beta}'' = \frac{b''}{\bar{K}_b} \cdot \xi^{-z_b/2} \tag{7}$$

$$V' = 1+\bar{\alpha}'+\bar{\beta}'+\bar{r}\bar{\alpha}'\bar{\beta}' \qquad V'' = 1+\bar{\alpha}''+\bar{\beta}''+\bar{r}\bar{\alpha}''\bar{\beta}''$$
$$R = R'\binom{o}{o}+\bar{\alpha}' \cdot R'\binom{a}{a}+\bar{\beta}'R\binom{b}{o}+\bar{r}\bar{\alpha}'\bar{\beta}'R'\binom{b}{a}$$

with

$$R'\tbinom{o}{o} = R'\tbinom{oo}{oo} + \bar{\alpha}''R\tbinom{oo}{oa} + \bar{\beta}''R\tbinom{ob}{oo} + \bar{r}\bar{\alpha}''\bar{\beta}''R\tbinom{ob}{oa} \qquad \text{etc.}$$

for $R'\tbinom{o}{a}$ etc.

$$R\tbinom{ob}{ao} = \frac{1}{x_T}\left(\frac{1}{\bar{\beta}'_a} + \frac{1}{\bar{\beta}''_b}\right) \qquad R\tbinom{ob}{oa} = \frac{1}{x_T}\left(\frac{1}{\bar{\beta}'_o} + \frac{1}{\bar{\beta}''_{ab}}\right)$$

The following "standard parameters" are defined.

$$(J'_a)_{max} = (1+\bar{r}\bar{\beta}')V''/R'(a) \qquad (K_M)_a = \bar{K}_a \cdot R'(o)/R'(a)$$

$$f_{max} = \frac{(1+\bar{r}\bar{\beta}')(1+\bar{\beta}'')}{(1+\bar{\beta}')(1+\bar{r}\bar{\beta}'')}$$

and for the special case $\bar{\alpha}''$; $\bar{\beta}'' \to o$

$$(J'_a)_{max} = (1+\bar{r}\bar{\beta}')/[R\tbinom{oo}{ao} + \bar{r}\bar{\beta}'R\tbinom{bo}{ao}]$$

$$(K_m)_a = \bar{K}_a \cdot \frac{R\tbinom{oo}{oo} + \bar{\beta}'R\tbinom{bo}{oo}}{R\tbinom{oo}{ao} + \bar{r}\bar{\beta}'R\tbinom{bo}{ao}}$$

$$f_{max} = \frac{1+\bar{r}\bar{\beta}'}{1+\bar{\beta}'}$$

REDUCED MODELS

The following reduced models will be considered in analogy to our previous treatment. (I) Affinity-type models, i.e. those in which the co-ion modifies only the affinity of the carrier for the transportee without changing its mobility coefficient. (II) Velocity-type models, i.e. those in which the co-ion modifies only the mobility of the carrier, without changing its affinity for the transportee. (III) Mixed-type models, i.e. those in which both effects of the co-substrate are combined.

In the previous paper, the two modifying effects have been handled as being either direct or quasiallosteric, so that each of the above types could be subdivided in a direct and a quasiallosteric variant. In the present paper, we shall only consider the direct effect, in terms of the conventional carrier model, because in the quasiallosteric variants the effect of electrical potential difference is quite similar. The effect of the membrane potential depends on the charge of the unloaded carrier, z. As mentioned above, however, we shall restrict ourselves to two alternatives, assuming that the empty carrier is either electrically neutral ($z = 0$) or bears one negative charge per Na^+ binding site ($z = -1$). In the first case, the ternary complex should be positively charged, in the second case, it should be neutral.

In order to implement the effects of electrical potentials in our equation we have to make some additional assumptions concerning the nature of the membrane and its function as an electrical and osmotic barrier.

1. The constant field approximation is valid, an assumption shown to be reasonable for many biological and artificial membranes. In other words, we assume that the electrical potential of charged particles within the membrane is a linear function of their distance from one membrane phase in direction to the other.

2 As to mediated transport we assume that the membrane is not homogeneous but permits penetrations by loaded or unloaded carrier species only by rapid "jumps" between the two membrane boundaries, where they are accessible to the solutes of the adjacent solutions These jumps are so rapid that practically all carrier species occur in the membrane boundaries only, whereas intermediate location within the membranes are negligible at any time. In order that the translocation step be rate limiting, as has been assumed above, these jumps have to occur infrequently, at least much less frequently than the interactions between solute and carrier in the boundaries. In other words, the penetration behaves like a process having a high activation energy, which is required to overcome the osmotic barrier

This assumption has some consequences for the kinetic equations describing the penetration of charged particles under the influence of an electrical field. For a homogeneous membrane, allowing continuous diffusion of charged particles, this equation looks as follows.

$$J = p \cdot \frac{\ln \xi}{\xi^{\frac{1}{2}} - \xi^{-\frac{1}{2}}} \cdot (c' \cdot \xi^{\frac{1}{2}} - c'' \xi^{-\frac{1}{2}})$$

If, however, the penetration occurs by jumps, this equation has to be expanded by a function of the hight and profile of the osmotic barrier. This expansion can be greatly simplified by the subsequent assumption

3. The barrier is assumed to be a single wall with a symmetrical triangular profile, located in the middle of the membrane phase. As to the height of this barrier we simply assume that it greatly exceeds the electrical potential difference. In other words, the activation energy required to overcome this barrier is much greater than the electrical potential difference across the membrane. Under these conditions, the probability that a charged particle jumps this barrier is proportional to the amount of charged particles within the membrane boundary times the Boltzmann coefficient For simplicity we assume that the height of the barrier is the same for all charged carrier species so that the above factor can be incorporated in the permeability coefficient. Otherwise any electrical function of the membrane can be treated as unity and the resulting equation will look as follows [6, 7]

$$J = p(c'\xi^{\frac{1}{2}} - c''\xi^{-\frac{1}{2}})$$

Since only the translocation of the charged species depends on the potential difference, we may conveniently place the electrical potential coefficients near the unidirectional rate coefficients.

$$J_i = p_i' \xi^{\frac{1}{2}} c' - p_i'' \xi^{-\frac{1}{2}} c_i''$$

We see that we simply have to multiply the rate coefficients of each charged species, be it the ternary complex or the empty carrier, respectively, by the appropriate electrical activity coefficient, whereas all electrically independent parameters of the barrier, being considered invariant, are incorporated in the p values. If the peak of the barrier was not located exactly in the middle of the membrane, the factors $\frac{1}{2}$ and $-\frac{1}{2}$, respectively, in the exponential functions would have to be replaced by n and $(n-1)$, respectively, n being the fractional distance of the barrier peak from the left face of the membrane. Since in the present context we merely intend to investigate the principles of the electric effects, we need not consider such details.

(I) Affinity-type models

The carrier (X) has two binding sites, one for the uncharged transportee (A) and another for the effector (B), bearing a charge +1. Binding of the effector to X increases the affinity to the transportee by the factor r and vice versa. At $\psi = 0$ the mobilities of the different carrier species are equal. The standard parameters of the model are the following:

$z = 0$

$$(J'_a)_{max} = x_T \cdot p \frac{1+r\beta'\xi^{+\frac{1}{2}}}{2+(1+\xi^{+\frac{1}{2}})r\beta'}$$

$$(K_m)_a = K_a \frac{2+(1+\xi^{+\frac{1}{2}})\beta'}{2+(1+\xi^{+\frac{1}{2}})\gamma\beta'}$$

$$f_{max} = \frac{1+r\beta'\xi^{+\frac{1}{2}}}{1+\beta'\xi^{+\frac{1}{2}}}$$

$z = -1$

$$(J'_a)_{max} = x_T \cdot p \frac{1+r\beta'\xi^{+\frac{1}{2}}}{(\xi^{-\frac{1}{2}}+\xi^{+\frac{1}{2}})+r\beta'(\xi^{+\frac{1}{2}}+1)}$$

$$(K_m)_a = K_a \frac{(\xi^{+\frac{1}{2}}+\xi^{-\frac{1}{2}})+\beta'(\xi^{+\frac{1}{2}}+1)}{(\xi^{+\frac{1}{2}}+\xi^{-\frac{1}{2}})+r\beta'(\xi^{+\frac{1}{2}}+1)}$$

$$f_{max} = \frac{1+r\beta'\xi^{+\frac{1}{2}}}{1+\beta'\xi^{+\frac{1}{2}}}$$

If the loaded carrier has a velocity different from that of the unloaded one, an assumption often made to explain trans effects, the standard parameters have the following form, $\rho_a = p_a/p_o = p_{ab}/p_b$

$z = 0$

$$(J'_a)_{max} = x_T \cdot p \frac{\rho_a(1+r\beta'\xi^{+\frac{1}{2}})}{(1+r\beta')+\rho_a(1+r\beta'\xi^{+\frac{1}{2}})}$$

$$K_m = K_a \frac{(1+\beta')+(1+\beta'\xi^{+\frac{1}{2}})}{(1+r\beta')+\rho_a(1+r\beta'\xi^{+\frac{1}{2}})}$$

$z = -1$

$$(J'_a)_{max} = x_T \cdot p \frac{\rho_a(1+r\beta'\xi^{+\frac{1}{2}})}{(1+r\beta')\xi^{+\frac{1}{2}}+\rho_a(\xi^{-\frac{1}{2}}+r\beta')}$$

$$K_m = K_a \frac{(1+\beta')\xi^{+\frac{1}{2}}+(\xi^{-\frac{1}{2}}+\beta')}{(1+r\beta')\xi^{+\frac{1}{2}}+\rho_a(\xi^{-\frac{1}{2}}+r\beta')}$$

II) Velocity-type models

Transportee and effector do not influence each other's binding. The velocities of the different carrier species differ from that of the unloaded carrier by a factor of ρ_a ρ_b ρ_{ab}, respectively. The standard parameters are:

$z = 0$

$$(J'_{\mathrm{a}})_{\max} = x_{\mathrm{T}}\, p_0 \frac{\rho_{\mathrm{a}} + \rho_{\mathrm{ab}} \xi^{\frac{1}{2}} \beta'}{(1+\rho_{\mathrm{a}}) + (1+\rho_{\mathrm{ab}} \xi^{\frac{1}{2}})\beta'}$$

$$K_{\mathrm{m}} = K_{\mathrm{a}} \frac{2+(1+\rho_{\mathrm{b}} \xi^{\frac{1}{2}})\beta'}{(1+\rho_{\mathrm{a}})+(1+\rho_{\mathrm{ab}} \xi^{\frac{1}{2}})\beta'}$$

$$f_{\max} = \frac{1}{\rho_{\mathrm{a}}} \; \frac{\rho_{\mathrm{a}} + \rho_{\mathrm{ab}} \xi^{\frac{1}{2}} \beta'}{1+\rho_{\mathrm{b}} \xi^{\frac{1}{2}} \beta'}$$

$z = -1$

$$(J'_{\mathrm{a}})_{\max} = x_{\mathrm{T}}\, p_0 \frac{\rho_{\mathrm{a}} + \rho_{\mathrm{ab}} \xi^{\frac{1}{2}} \beta'}{(\xi^{\frac{1}{2}} + \rho_{\mathrm{a}} \xi^{-\frac{1}{2}}) + (\xi^{\frac{1}{2}} + \rho_{\mathrm{ab}})\beta'}$$

$$K_{\mathrm{m}} = K_{\mathrm{a}} \frac{(\xi^{\frac{1}{2}} + \xi^{-\frac{1}{2}}) + (\xi^{\frac{1}{2}} + \rho_{\mathrm{b}})\beta'}{(\xi^{\frac{1}{2}} + \rho_{\mathrm{a}} \xi^{\frac{1}{2}}) + (\xi^{\frac{1}{2}} + \rho_{\mathrm{ab}})\beta'}$$

$$f_{\max} = \frac{1}{\rho_{\mathrm{a}}} \cdot \frac{\rho_{\mathrm{a}} + \rho_{\mathrm{ab}} \cdot \xi^{\frac{1}{2}} \beta'}{1+\rho_{\mathrm{b}} \xi^{\frac{1}{2}} \beta'}$$

(III) Mixed-type models

Combination of transportee A and effector B enhances the velocity of the carrier by factor of ρ_{a} and ρ_{b}, respectively. The binding of the effector increases the affinity of the carrier to the transportee by a factor of r and vice versa. The standard parameters are the following:

$z = 0$

$$(J'_{\mathrm{a}})_{\max} = x_{\mathrm{T}} \cdot p_0 \frac{\rho_{\mathrm{a}}(1+\rho_{\mathrm{b}} r \xi^{\frac{1}{2}} \beta\ )}{(1+\rho_{\mathrm{a}}) + (1+\rho_{\mathrm{a}} \cdot \rho_{\mathrm{b}} \xi^{\frac{1}{2}}) r \beta'}$$

$$K_{\mathrm{m}} = K_{\mathrm{a}} \frac{2+(1+\rho_{\mathrm{b}} \xi^{\frac{1}{2}})\beta'}{(1+\rho_{\mathrm{a}}) + (1+\rho_{\mathrm{a}} \rho_{\mathrm{b}}\ \ \xi^{\frac{1}{2}}) r \beta'}$$

$$f_{\max} = \frac{1+\rho_{\mathrm{b}} r \xi^{\frac{1}{2}}\ \ \beta'}{1+\rho_{\mathrm{b}} \xi^{\frac{1}{2}} \beta'}$$

$z = -1$

$$(J'_{\mathrm{a}})_{\mathrm{ma}} = x_{\mathrm{T}} \cdot p_0 \frac{\rho_{\mathrm{a}}(1+\rho_{\mathrm{b}} r \xi^{\frac{1}{2}} \beta')}{(\xi^{\frac{1}{2}} + \rho_{\mathrm{a}} \xi^{-\frac{1}{2}}) + (\xi^{\frac{1}{2}} + \rho_{\mathrm{a}} \rho_{\mathrm{b}}) r \beta'}$$

$$K_{\mathrm{m}} = K_{\mathrm{a}} \frac{(\xi^{\frac{1}{2}} + \xi^{-\frac{1}{2}}) - (\xi^{\frac{1}{2}} + \rho_{\mathrm{b}})\beta'}{(\xi^{\frac{1}{2}} + \rho_{\mathrm{a}} \xi^{-\frac{1}{2}}) + (\xi^{\frac{1}{2}} + \rho_{\mathrm{a}} \rho_{\mathrm{b}}) r \beta'}$$

DISCUSSION

As has been stated in the introduction, a chemical potential gradient and an electrical potential gradient are thermodynamically fully equivalent and mutually interchangeable as driving forces of ion-linked transport processes. The question arises whether the same is necessarily true kinetically, i.e. for the transport rates resulting from such driving forces. In other words, are these rates the same whether a given driving force results from a difference in concentration or from an equivalent difference in electrical potential?

The kinetics of transport rates are usually based on the law of mass action, the rates being treated as being proportional to concentrations or electrochemical activities, rather than to thermodynamic potential differences, of the species' involved. Accordingly, the equations derived above represent the overall transport rates as functions of the difference in electrochemical activity of the driving ion species B, between the two sides of the barrier:

$$J_a = f(\Delta\bar{\beta})$$

An electrical potential difference ψ, if present, appears thus not as such but implicitly in the corresponding electrochemical activity coefficient. In the literature, this coefficient is being handled in different ways. Often only one of the two concentrations is multiplied by such a coefficient, which in that case refers to the total potential difference $(\beta'_+\xi^+ - \beta''_+)$. The electrical potential on the ''side is then taken as the reference potential and set arbitrarily equal zero, so that the coefficient of β'' becomes unity. In the present paper, however, we prefer, for reasons given above, the other treatment, in which each of the two concentrations is multiplied by the appropriate coefficient, each of which refers to only one half of the total potential $(\beta'_+\xi^{\frac{1}{2}} - \beta''_+\xi^{-\frac{1}{2}})$. In this case the electrical potential in the middle of the membrane is taken to be the reference potential and set equal to zero. Fundamentally, both treatments are equivalent. In the present kinetic derivations, however, there is some minor difference as will be shown farther below. The reason for this difference is that in the former treatment the electrochemical potential of the ion on the trans side is being fixed whereas that on the cis side is varied. In the latter treatment, on the other hand, both electrochemical activities are changed reciprocally, so that the geometric mean of both remains constant This will have to be taken into consideration if electrical and osmotical effects on $\Delta\bar{\beta}$ are to be compared.

Obviously, a given change of that difference can be produced in various ways e.g. osmotically by a difference in concentration of B (β), and electrically, by an electrical potential difference across the membrane. To compare the effects on J_a of these differences with each other we study each separately, selecting two borderline cases in which given differences in electrochemical activity of B are produced either osmotically or electrically. In the case of an electrical change it is important to know the electrical charge of the translocated species', especially of the ternary complex and the unloaded carrier. For reason of simplicity we have considered only the following two alternatives:

1. The empty carrier is neutral ($z = 0$), so that the ternary complex has to be positively charged, and 2. the empty carrier is negative ($z = -1$), the ternary complex being neutral. All these conditions are tested separately for both the affinity type and velocity type model.

First we calculated the transport rates under the various possible conditions, uniformly for low concentrations of A. The results are shown in Fig. 1 The replacement of a concentration difference by an equivalent electrical term $\beta(\xi^{\frac{1}{2}} - \xi^{-\frac{1}{2}})$, under the condition that $z = 0$, lowers the resulting transport rate as if kinetically an electri-

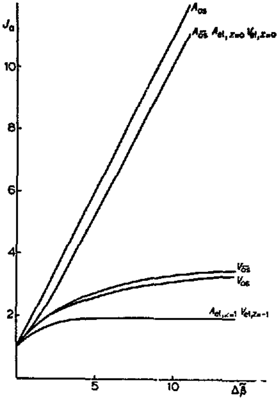

Fig 1 The transport rate of main solute, J_a, in response to a difference in electrochemical potential of the driving ion, B. Ordinate J_a at low concentration of A ($\alpha' \rightarrow 0$) in relative units, the transport in the absence of an electrochemical activity difference of B taken as unity Abscissa: The difference in electrochemical activity of B ($\tilde{\beta}' - \tilde{\beta}''$), also in relative units, the transconcentration of B, β'' taken as unity (except for curve $A_{\overline{os}}$) in which the geometric mean of $\tilde{\beta}'$ and $\tilde{\beta}''$ is taken as unity The curves labeled by A refer to the affinity type model, those labeled by V, to the velocity type model The subscript os is to indicate, that the difference in electrochemical activity of B is due to a change in concentration only, i e. in the absence of an electrical potential. The subscript el is to indicate that the difference in electrochemical activity of B is due to an electrical potential difference only, i.e in the absence of a concentration difference. As indicated, these latter curves refer either to a neutral ($z = 0$), or a negatively charged ($z = -1$) empty carrier For simplicity reason, it is assumed that in the affinity type model the binary complexes XA and XB are practically nonexisting owing to extremely low affinity Analogously for the velocity type the contributions of the rates of the above mentioned binary complexes are also neglected, assuming that their translocation across the barrier is too low The ratio (ρ) of the mobility of the ternary complex, p_{ab}, over that of the empty carrier, p_0, is uniformly 2 The transconcentration of A, α'', is 0 in all cases

cal potential difference were less effective than an equivalent chemical driving force. This conclusion is, however, not justified, because the difference would vanish if we had introduced the potential change only by changing the electrochemical activity coefficient of the first concentration, leaving the electrochemical activity on the other side constant Since, as mentioned above, in the present paper we have changed the electrochemical activities while leaving the geometric mean of both constant, we should for a comparison do the same if we introduce a concentration difference, namely leave the geometric mean of the concentrations constant. If we do this, as illustrated by curve $A_{\overline{os}}$, the two curves for $A_{\overline{os}}$ and $A_{el;z=0}$ coincide. In other words, electrical and osmotic changes of the electrochemical activities on both sides of the membrane are equivalent to each other, as far as the affinity type is concerned, and provided that $z = 0$. In either case, however, the resulting transport rates are higher if $\Delta\beta$ is obtained by keeping β'' constant rather than by keeping the geometric mean of β'' constant, probably because in the former case the resistance is lower.

If we replace the neutral carrier ($z = 0$) by a negative one ($z = -1$), the transport rate of A considerably decreases for the same difference in electrochemical activity ($\Delta\beta$) of B. We may conclude that the same electrical potential difference is less effective, if it acts on the transport only indirectly, namely via the empty carrier rather than via the ternary complex ($A_{el; z=-1}$).

For the velocity type model it makes apparently little difference whether the erection of a concentration difference leaves the geometric mean of the concentration of B constant or not (V_{os}, $V_{\bar{os}}$).

If the concentration difference is replaced by an equivalent difference in electrical potential difference, the response of the transport rate in velocity type model depends on the charge of the carrier, z. If $z = 0$, i.e. if the ternary complex is positive the same transport rate appears to respond more strongly to an electrical p d than to an equivalent concentration difference. The opposite is true if $z = -1$, the ternary complex being neutral, as is seen from curve $V_{el, z=-1}$.

The behavior of the so-called standard parameters of the flow of A under the various conditions is presented in Fig. 2 and 3, showing the maximum transport rates, (J_{max}), and the apparent Michaelis constants, (K_m), respectively. Again, the abscissa show the differences in electrochemical activity, which may be produced either osmotically, by a change in concentration difference of driver ion (A_{os}; V_{os}) or electri-

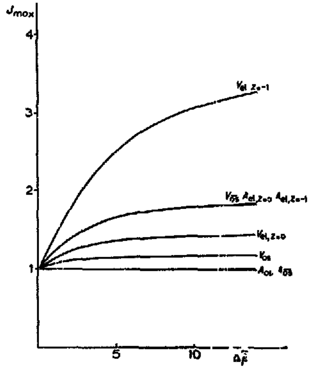

Fig 2. The maximum velocity of transport of A, $(J_a)_{max}$, in response to variation of the electrochemical activity difference of B Ordinate: $(J_a)_{max}$ in relative units, the value obtained on the absence of an electrochemical activity difference of B taken to be unity Abscissa labeling of the curve and the underlying consumption concerning the binary complexes are the same as in Fig. 1 The transconcentration of A, α'', is 0 under all conditions.

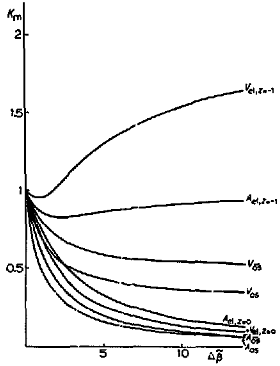

Fig 3 The Michaelis Constant of the transport of A as a function of electrochemical potential difference of B The ordinate is K_m relative units, the value in the absence of an electrochemical activity difference of B is taken to be unity. The abscissa, the labeling of the curves and the underlying assumptions concerning the binary complexes of the carrier are the same as in the previous figures. The transconcentration of A, α'', is 0 under all conditions.

cally, by the appropriate change of the electrical potential difference, the latter under the two alternative conditions, $z = 0$ ($A_{el,z=0}$ and $V_{el,z=0}$) or $z = -1$ ($A_{el,z=-1}$ and $V_{el,z=-1}$). The various combinations and the corresponding changes in standard parameters are listed in Table I. If a given difference in electrochemical activity of B is produced osmotically only, i e in the absence of an electrical potential difference, the

TABLE I

RESPONSE OF J_{max} AND K_m OF MAIN-SOLUTE (A) TRANSPORT TO OSMOTIC AND ELECTRICAL CHANGES IN ELECTROCHEMICAL ACTIVITY OF B

Difference in electrochemical activity ($\Delta\tilde{\beta}$)	J_{max}[a]	K_m[a]
Affinity type		
osmotic ($\Delta\Psi = 0$)	–	↓↓
electric ($\Delta\beta = 0$)		
$z = 0$	↑↑	↓
$z = -1$	↑↑	–
Velocity type		
osmotic ($\Delta\Psi = 0$)	↑	↓
electric ($\Delta\beta = 0$)		
$z = 0$	↑	↓↓
$z = -1$	↑↑↑	↑↑

response of the standard parameters is the same as has already been described in the previous paper. The affinity type shows only a decrease in K_m, and the velocity type, an increase in J_m, the other parameters remaining largely unchanged (curves A_1 and V_1 in the corresponding figures). Such responses, however, are different, if equivalent differences in electrochemical activity of the driver ion are produced electrically only.

With the affinity type model there appears an increase in J_m, no matter whether $z = 0$ or $z = -1$. In other words, the pure affinity type has adopted a feature which was previously considered typical for the velocity type.

The response of K_m to an electrical potential difference, contrary to that of J_m, with this type depends strongly on z, i.e. on whether or not the empty carrier bears a negative charge. If $z = 0$, K_m strongly decreases with increasing potential difference, as it does with increasing concentration difference. If $z = -1$, K_m is essentially insensitive towards changes in electrical potential differences.

With the velocity type an electrical potential difference, replacing an equivalent concentration difference, generally increases J_{max}. This effect depends on z only if the mobilities of ternary complex is different from that of the empty carrier. If, for instance, the loaded carrier translocates more readily than the empty one, the electrical potential difference increases J_m much more strongly at $z = -1$ than at $z = 0$ (Fig. 3, curve $V_{el, z=0}$; $V_{el, z=-1}$). As to K_m, it is also changed by an electrical potential difference, a feature previously considered typical for the affinity type. The sense of change depends on z.

Since an electrical potential difference across a membrane affects only the rates of translocation but not the binding and releasing reactions between carrier and solutes in the interfaces, it can be anticipated that the effects of the potential difference vanish to the extent that the latter reactions become rate limiting for the overall transport. At the same time, however, the effects of co-transport will also vanish, since these also depend on the extent to which the translocation steps are limiting, as has been demonstrated previously [3]. If, for instance, the overall transport rate were entirely determined by the binding and release reactions between solutes and carrier, the coupling between solute A and solute B would no longer be energetic, only catalytic, i.e. A could no longer be moved thermodynamically uphill by a downhill gradient of solute B. Hence, such situations are not relevant in the present context and need therefore not be analysed with respect to electrical potential difference effects.

As has been stated initially, in the present paper only simplified, extreme conditions are tested, so the difference in electrochemical activity of the driver ions has been changed either osmotically only, or electrically only. In reality, both electrical potential and concentration difference may change simultaneously in some cases. This makes the picture more complicated, but it may approximately be anticipated, that electrical and concentration effects are largely additive, so that the overall effect may be predicted at least qualitatively. On the other hand, it seems feasible that with selected systems experimental conditions can be held such as to study electrical and concentration effects separately. The difference between different model types appear to be so great that a distinction between alternative models and carrier charges may be elucidated experimentally on the basis of predictions derived from equations such as those predicted in the present paper.

The above derivations are merely kinetical and therefore based on the law of mass action, the translocational flow of each species being treated as a linear function of the difference in electrochemical activity of that species. The interesting question arises how the electrical potential difference will affect the treatment of co-transport if the latter is expressed in terms of thermodynamics of irreversible processes. In any notation of the thermodynamics of irreversible processes the driving force for each charged species, X, incorporates both the chemical and the electrical potential difference as completely equivalent and interchangeable forces. Hence any differential effects of these two forces on the overall transport rate, as should be anticipated in view of the above derivations can only appear in the coefficients, L. This dependence of these coefficients on the contribution of electrical potential difference to the driving force will be dealt with in a subsequent paper

GLOSSARY

a, b	concentrations of A and B in the bulk phases
x, ax, bx, abx	concentrations of free carrier, carrier-A-, carrier-B-complex and ternary complex in the boundaries of the transport region
x_T	total concentration of carrier in the transport region
K_a, K_b	dissociation constants of carrier-A-and carrier-B-complex
$\bar{K}_a, \bar{K}_b$	side independent apparent dissociation constants
r	factor, by which the affinity of the carrier for A is changed by B and vice versa
p_i	mobilities of the different carrier species
z, z_A, z_B	electrical charges of free carrier, A and B
$V, R, R\binom{i}{j}$	polynoma in α and β defined by Eqn. 7
J_a, J_b	unidirectional fluxes of A and B
$(J_a)_{max}$	maximum velocity
K_m	Michaelis constant
f_{max}	maximal accumulation ratio of A
α, β	relative chemical activities of A and B referred to the dissociation constants K_a and K_b, respectively
$\bar{\alpha}, \bar{\beta}$	relative electrochemical activities of A and B referred to the side independent apparent dissociation constants
$\rho_a, \rho_b, \rho_{ab}$	factors by which the mobilities of the different carrier species differ from that of the free carrier
ξ	electrical activity coefficient
ψ	membrane potential

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