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# HOW THE KINETIC PARAMETERS OF THE SIMPLE CARRIER ARE AFFECTED BY AN APPLIED VOLTAGE

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## Summary

The kinetic parameters ($K$, $R_{oo}$, $R_{12}$, $R_{21}$ and $R_{ee}$) of the simple carrier model are analysed as a function of applied voltage for various cases of charged substrates and carriers. If certain parameters are invariant with voltage, strong conclusions can be made as to the charge on the free carrier. In one particular case (where the parameter $R_{oo}$ is invariant while $R_{12}$ and $R_{21}$ display a non-linear dependence on the exponential of the voltage) it may be possible to identify the isomerisation of the carrier-substrate complex.

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We have recently shown, in great detail, how a system which behaves as a simple carrier can be treated, and characterised in terms of a number of kinetic parameters which completely describe the steady-state behaviour of the system [1]. These parameters are an affinity, $K$, and four resistances, these latter determining the maximum velocity of transport by the carrier in certain defined experimental situations. In brief, any unidirectional flux of substrate S can be described by the equation

$$v_{1\to 2} = \frac{Ke^{zu/2}S_1 + S_1S_2}{K^2R_{oo} + KR_{12}e^{zu/2}S_1 + KR_{21}e^{-zu/2}S_2 + R_{ee}S_1S_2}$$

where $v_{1\to 2}$ is the unidirectional flux of S in the direction 1 to 2, $K$ is the affinity parameter, $R_{12}$, $R_{21}$, $R_{ee}$ and $R_{oo}$ are the resistances while $S_1$ is the concentration of substrate at side 1 and $S_2$ that at side 2 of the membrane. The substrate carries a net charge of $z$, while the term $u$ is the "reduced transmembrane electrical potential difference", given by $u = (F/RT)(\psi_1 - \psi_2)$, where $F$ is the Faraday and $R$ the gas constant, $T$ the absolute temperature and $(\psi_1 - \psi_2)$ is the electrical potential difference between sides 1 and 2 of the membrane. The interpretation of these kinetic parameters in terms of two particular models for carrier-mediated diffusion is given in Table I, the models themselves being depicted as Figs. 1 and 2. Fig. 1 is the conventional carrier model, Fig. 2 the

TABLE I

INTERPRETATION OF THE KINETIC PARAMETERS FOR THE CARRIER MODEL IN TERMS OF THE RATE CONSTANTS (modified from Lieb and Stein [1])

| Parameter (per mol of carrier) | Model of Fig. 1 (two forms of complex) | Model of Fig. 2 (one form of complex) |
|---|---|---|
| $K$ | $\frac{k_1}{f_1} e^{zu/2} + \frac{k_2}{f_2} e^{-zu/2} + \frac{k_2 b_2}{f_2 g_2} e^{-zu/2}$ | $\frac{k_1}{f_1} e^{zu/2} + \frac{k_2}{f_2} e^{-zu/2}$ |
| $R_{oo}$ | $\frac{1}{k_1} + \frac{1}{k_2}$ | $\frac{1}{k_1} + \frac{1}{k_2}$ |
| $R_{12}$ | $\frac{1}{b_2} + \frac{1}{k_2} + \frac{1}{g_1} + \frac{g_2}{g_1 b_2}$ | $\frac{1}{b_2} + \frac{1}{k_2}$ |
| $R_{21}$ | $\frac{1}{b_1} + \frac{1}{k_1} + \frac{1}{g_2} + \frac{g_1}{g_2 b_1}$ | $\frac{1}{b_1} + \frac{1}{k_1}$ |
| $R_{ee}$ | $\frac{1}{b_1} + \frac{1}{b_2} + \frac{1}{g_1} + \frac{1}{g_2} + \frac{g_2}{g_1 b_2} + \frac{g_1}{g_2 b_1}$ | $\frac{1}{b_1} + \frac{1}{b_2}$ |

"necessarily simplified" form [2], where the transformation between those forms of the carrier that have bound substrate is suppressed, this isomerisation not being identifiable by steady-state measurements at a particular voltage.

In this present note, I show that if these defining parameters can be obtained as different voltages are applied across the membrane, so that they can be plotted as a function of the applied voltage, certain useful inferences can be drawn as to the state of electric charge of the carrier or carrier-substrate complex. In certain favourable circumstances it can even be possible to distinguish between the two models of Figs. 1 or 2.

My approach is as follows: I consider in turn each rate constant of the models of Figs. 1 and 2 and show how these are likely to be affected by the voltage applied across the membrane. Different cases will be considered, systematically, according as to the state of charge of the carrier or of the carrier-substrate complex. Each kinetic parameter is then considered in terms of the interpretations of Table I and a table constructed showing how each parameter for this particular case of model and charge characteristic varies with voltage.

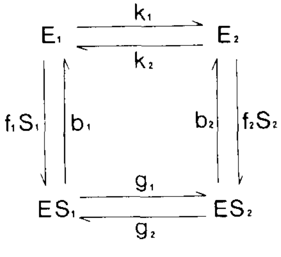


Fig. 1.

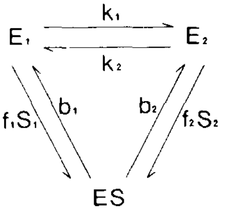


Fig. 2.

Two important assumptions need to be made over and above those made in the defining paper [1] of this series:

(i) Only those rate constants which are concerned in a flow across the membrane will be affected by the prevailing applied potential difference. That is, in Fig. 1, only the terms in $k$ and $g$ can be affected by the voltage while in Fig. 2 all terms can be so affected. Of course, only if the carrier species E and ES is charged will the applied voltage affect the relevant rate constant. Second-order effects, such as the effect of the applied potential on any residual dipoles that may remain in an uncharged species E or ES, will be ignored.

(ii) It is also necessary to confront the problem of the distribution of the electric field across the membrane, this being of course, an unknown. Since we are discussing the effect of the electric field on the rate constants for movement of the carrier in its various forms, we are really considering how the free energy of the various forms of the carrier and of the transition state for the interconversion between particular forms ($E_1 \rightleftharpoons E_2$ and $ES_1 \rightleftharpoons ES_2$) are differentially affected by the applied voltage. The approach used here is to consider various possible limiting cases (and intermediate cases) for the shape of the electric field. In one limiting case, I assume that the whole potential drop occurs between the relevant transition state and the form $E_1$ or $ES_1$ (in Fig. 1). Then only the rate constants for the $1 \rightarrow 2$ direction will be potential dependent, since the $E_2$ and $ES_2$ forms and their relevant transition states will be equipotential. In the other limiting case, only the rate constant for the $2 \rightarrow 1$ direction will be affected. I take also the intermediate case where the transition state is in the exact middle of the potential drop. Then the $1 \rightarrow 2$ and the $2 \rightarrow 1$ rate constants are equally and oppositely affected by the applied potential. For completeness, I consider also two other cases where the position of the transition state for the $ES_1 \rightleftharpoons ES_2$ transformation differs from that for the $E_1 \rightleftharpoons E_2$ transformation, with respect to the electric field. I consider here two limiting cases, one where $E_1$ and its transition state are at equipotential while it is $ES_2$ and its transition state that are at a different equipotential, and also the corresponding other limiting case where it is $E_2$ and its transition state and $ES_1$ and its transition state that are at their respective equipotentials. Taking all these cases in turn gives a clear picture of the overall phenomenology under all possible electrical potential profiles.

I will consider Figs. 1 and 2 together at first, in a general treatment and then go on to discuss them in detail, separately, since the model of Fig. 2 demonstrates some special difficulties.

We have four cases to consider:

*Case I.* The substrate is uncharged, the carrier is charged. Then the carrier-substrate complex bears the same charge as the free carrier. In Fig. 1, the rate constants in $k$ and $g$ will be equally affected by voltage, in Fig. 2 all steps may be affected by voltage.

*Case II.* The substrate is charged, the carrier is uncharged. Then the carrier-substrate complex bears the same charge as the free substrate. The rate constants $k$ in both figures are invariant with the applied voltage.

*Case III.* The substrate and free carrier are oppositely charged. Then the carrier-substrate complex is uncharged. Only the rate constants in $k$ vary, all others being invariant.

*Case IV.* The substrate and the free carrier possess the same charge. Then the carrier-substrate complex possesses two charges. The rate constants in $g$ in Fig.1 will be more steeply dependent on voltage than those in $k$. In Fig. 2, terms in $f$ and $b$ may be more steeply affected by voltage than the $k$ terms, as I shall show.

For each case in turn, for the model of Fig. 1 we will have to consider five subcases covering the particular assumptions made above, as to the shape of the electric field. For the model of Fig. 2, a less detailed analysis is given.

If a particular rate constant, say $k_1$, is affected by the applied potential, I will write $k_1 = k_1^* e^{u/2}$ (or, $k_1^* e^{-u/2}$. . . etc.), when $k_1^*$ is the value of the rate constant at zero transmembrane potential difference and the exponential term describes how the rate constant varies with the potential.

The parameters in $K$ and $R$ will be differentiated with respect to $e^{u/2}$, or $e^{-u/2}$ or to some higher power as convenient (and as will be clear from the text) and the derivative written as $\dot{K}$ or $\dot{R}$.

For the following discussion, the free carrier has unit positive charge. The carrier-substrated complex has none, one or two positive charges. For negatively charged cases, one merely reverses the relevant signs.

*Analysis of Fig. 1, two forms of the carrier-substrate complex*

On the basis of the above definitions one can write:

*Case I.* $k$ and $g$ both affected by voltage.

Subcase i. Rate constants in the two directions equally and oppositely affected. Then:

$$k_1 = k_1^* e^{u/2}; \quad k_2 = k_2^* e^{-u/2}; \quad g_1^* = e^{u/2}; \quad g_2 = g_2^* e^{-u/2}$$

from Table I,

$$K = \frac{k_1^* e^{u/2}}{f_1} + \frac{k_2^* e^{-u/2}}{f_2} + \frac{k_2^* b_2}{f_2 g_2^*} \qquad \dot{K} = \frac{k_1^*}{f_1} - \frac{k_2^* e^{-u}}{f_2}$$

$$R_{oo} = \frac{e^{-u/2}}{k_1^*} + \frac{e^{u/2}}{k_2^*} \qquad \dot{R}_{oo} = \frac{-e^{-u}}{k_1^*} + \frac{1}{k_2^*}$$

$$R_{12} = \frac{1}{b_2} + \frac{e^{u/2}}{k_1^*} + \frac{e^{-u/2}}{g_1^*} + \frac{g_2^* e^{-u}}{g_1^* b_2} \qquad \dot{R}_{12} = \frac{1}{k_2^*} - \frac{e^{-u}}{g_1^*} - \frac{2 g_2^* e^{-3u/2}}{g_1^* b_2}$$

$$R_{21} = \frac{1}{b_1} + \frac{e^{-u/2}}{k_1^*} + \frac{e^{u/2}}{g_2^*} + \frac{g_1^* e^{u}}{g_2^* b_1} \qquad \dot{R}_{21} = \frac{-e^{-u}}{k_1^*} + \frac{1}{g_2^*} + \frac{2 g_1^* e^{u/2}}{g_1^* b_1}$$

$$R_{ee} = \frac{1}{b_1} + \frac{1}{b_2} + \frac{e^{-u/2}}{g_1^*} + \frac{e^{u/2}}{g_2^*} + \frac{g_2^* e^{-u}}{g_1^* b_2} + \frac{g_1^* e^{u}}{g_2^* b_1} \qquad \dot{R}_{ee} = \frac{1}{g_2^*} - \frac{e^{-u}}{g_1^*} - \frac{2 g_2^* e^{-3u/2}}{g_1^* b_2} + \frac{2 g_1^* e^{u/2}}{g_2^* b_1}$$

For all these parameters, for this subcase, the dependence on the applied voltage is not simple. The parameters are neither invariant nor depend in a linear fashion on the applied voltage, in contrast to a number of situations that will be considered later. The results are recorded in Table II with the description "non-linear" as characterising the voltage dependence.

Subcase ii. Only $k_1$ and $g_1$ are affected by the applied voltage. Then: $k_1 = k_1^* e^u$ and $g_1 = g_1^* e^u$.

TABLE II

EFFECTS OF AN APPLIED VOLTAGE ON THE KINETIC PARAMETERS OF A CARRIER ON THE MODEL OF FIG. 1, WHERE THE $ES_1 \rightleftharpoons ES_2$ ISOMERISATION IS EXPLICITLY ASSUMED

The results collected in this table were obtained as described in the text. Where the results for any parameter are the same for all subcases considered, only a single entry is recorded. "linear" indicates that a linear dependence of the particular parameter on voltage is predicted, if the value of the parameter is plotted against the quantity given in the associated bracket.

| | Case and description | Subcase | Effect on | | | | |
|---|---|---|---|---|---|---|---|
| | | | $K$ | $R_{oo}$ | $R_{12}$ | $R_{21}$ | $R_{ee}$ |
| I | E and ES similar charge | i | non-linear | non-linear | non-linear | non-linear | |
| | (S uncharged) | ii or v | linear ($e^{u}$) | linear ($e^{-u}$) | linear ($e^{-u}$) | non-linear | non-linear |
| | | iii or iv | linear ($e^{-u}$) | linear ($e^{u}$) | non-linear | linear ($e^{u}$) | |
| II | E no charge, ES charged | i | | | non-linear | non-linear | |
| | (S charged) | ii or iv | non-linear | Invariant | linear ($e^{-u}$) | linear ($e^{u}$) | non-linear |
| | | iii or v | | | linear ($e^{-u}$) | linear ($e^{u}$) | |
| III | E charged, ES no charge | i | Invariant | non-linear | linear ($e^{u/2}$) | linear ($e^{-u/2}$) | |
| | (S and E oppositely | ii or v | linear ($e^{u/2}$) | linear ($e^{-u}$) | Invariant | linear ($e^{-u}$) | Invariant |
| | charged) | iii or iv | linear ($e^{-u/2}$) | linear ($e^{u}$) | linear ($e^{u}$) | Invariant | |
| IV | E charged, ES doubly | i | | non-linear | non-linear | non-linear | |
| | charged (S and E | ii or v | non-linear | linear ($e^{-u}$) | linear ($e^{-2u}$) | non-linear | non-linear |
| | similarly charged) | iii or iv | | linear ($e^{u}$) | non-linear | linear ($e^{2u}$) | |

Now, on substituting in Table I, and differentiating with respect to $e^u$ and $e^{-u}$:

| | |
|---|---|
| $\dot{K} = k_1^*/f_1$ | linear on $e^u$ |
| $\dot{R}_{oo} = 1/k_1^*$ | linear on $e^{-u}$ |
| $\dot{R}_{12} = 1/g_1^* + g_2/g_1^* b_2$ | linear on $e^{-u}$ |
| $\dot{R}_{21} = 1/k_1^* + g_1^* e^{2u}/g_2 b_1$ | non-linear |
| $\dot{R}_{ee} = 1/g_1^* + g_2/g_1^* b_2 - g_1^* e^{2u}/g_2 b_1$ | non-linear |

Subcase iii. Only $k_2$ and $g_2$ affected by the applied voltage. Then: $k_2 = k_2^* e^{-u}$ and $g_2 = g_2^* e^{-u}$.

Subcase iv. $k_2$ and $g_1$ only affected by voltage (oppositely). Then: $k_2 = k_2^* e^{-u}$ and $g_1 = g_1^* e^u$.

Subcase v. $k_1$ and $g_2$ only affected. Then: $k_1 = k_1^* e^u$ and $g_2 = g_2^* e^{-u}$.

The results obtained, on substituting in Table I and performing the appropriate differentiation are collected in Table II.

*Case II.* Only $g$ being affected by voltage.

Subcase i. $g_1 = g_1^* e^{u/2}$; $g_2 = g_2^* e^{-u/2}$. Others invariant.

$$K = \frac{k_1}{f_1} + \frac{k_2}{f_2} + \frac{k_2 b_2 e^{u/2}}{f_2 g_2^*} \qquad \dot{K} = \frac{k_2 b_2}{f_2 g_2^*}$$

$$R_{oo} = \frac{1}{k_1} + \frac{1}{k_2} \qquad \dot{R}_{oo} = 0$$

$R_{12}$, $R_{21}$ and $R_{ee}$ depend on the voltage in a complex fashion, but it is clear that $R_{oo}$ is invariant with the applied voltage while $K$ depends linearly on the applied voltage, if plotted against $e^{u/2}$.

Subcase ii. $g_1 = g_1^* e^u$.

Subcase iii. $g_2 = g_2^* e^{-u}$.

Subcase iv. Identical with subcase ii.

Subcase v. Identical with subcase iii and the results are collected in Table II.

*Case III.* Only $k$ being affected by voltage.

Subcase i. $k_1 = k_1^* e^{u/2}$; $k_2 = k_2^* e^{-u/2}$. Others invariant.

$\dot{R}_{12} = \dfrac{1}{k_2^*}$ and hence $R_{12}$ is linear with $e^{u/2}$.

$R_{21} = \dfrac{1}{b_1} + \dfrac{e^{-u/2}}{k_1^*} + \dfrac{1}{g_2} + \dfrac{g_1}{g_2 b_1}$ and hence $R_{21}$ is linear with $e^{-u/2}$.

$\dot{R}_{ee} = 0$ and hence $R_{ee}$ is invariant with the applied voltage.

Subcases ii and v. $k_1 = k_1^* e^u$.

Subcases iii and iv. $k_2 = k_2^* e^{-u}$ and the results are collected in Table II.

*Case IV.* $k$ affected by voltage, $g$ doubly affected by voltage.

Subcase i. $k_1 = k_1^* e^{u/2}$; $k_2 = k_2^* e^{-u/2}$; $g_1 = g_1^* e^u$; $g_2 = g_2^* e^{-u}$, since the complex possesses two unit charges.

Subcase ii. $k_1 = k_1^* e^u$; $g_1 = g_1^* e^{2u}$.

Subcase iii. $k_2 = k_2^* e^{-u}$; $g_2 = g_2^* e^{-2u}$.

Subcase iv. $k_2 = k_2^* e^{-u}; g_1 = g_1^* e^{2u}$.

Subcase v. $k_1 = k_1^* e^{u}$; $g_2 = g_2^* e^{-2u}$ and the results are collected in Table II.

*Analysis of Fig. 2, one form of the carrier-substrate complex*

The problem here is that the rate constant in $f$ and $b$, on the particular assumptions of this model, contain steps that may be more or less unaffected by voltage. It is difficult to be sure how these rate constants will be affected by the applied voltage. In order to make the essential points of the analysis, however, it is sufficient to confine our discussion to the particular subcase i considered above (there, for the model of Fig. 1), where the transition state for a particular transformation coincided exactly with the mid-point of the electric field. On this particular subcase, we will now treat separately two limiting situations and a defined intermediate situation, according as to which transition state is the one that is at the centre of the electric field.

It could be that the step labelled as $f_1$ in Fig. 2 indeed takes the carrier-substrate complex completely across that portion of the membrane where the difference of electrical potential is applied, so that the transition state of this transformation is at the centre of the electric field. In this case, both $f_1$ and $b_1$ will be affected by the applied voltage to the same extent as are $k_1$ and $k_2$, respectively, while $f_2$ and $b_2$ will be unaffected. The other extreme possibility is that only $f_2$ and $b_2$ are affected by the electric field while $f_1$ and $b_1$ are unaffected. Then $b_2$ and $k_1$ will be affected to the same extent, as will $f_2$ and $k_2$. The intermediate case will be that the step $f_1$ takes the carrier complex to the exact middle of the electric field, $b_2$ taking the complex the remainder of the distance. In this case each step is affected by one-half the full value of the potential difference and terms such as $e^{u/4}$ or $e^{-u/4}$ will enter into the relations defining the rate constants. I treat Case I with steps $f_1$ and $b_1$ being affected by the full value of the potential in detail. These, together with the other results derived in similar fashion, are collected in Table III.

*Case I.* With $f_1$ and $b_1$ affected by the whole of the potential gradient. $k_1 = k_1^* e^{u/2}; k_2 = k_2^* e^{-u/2}; f_1 = f_1^* e^{u/2}; b_1 = b_1^* e^{-u/2}$

$$K = \frac{k_1}{f_1^*} + \frac{k_2^* e^{-u/2}}{f_2} \qquad \dot{K} \text{ (with respect to } e^{-u/2}) = \frac{k_2^*}{f_2^*}$$

$$R_{oo} = \frac{e^{-u/2}}{k_1^*} + \frac{e^{u/2}}{k_2^*} \qquad \dot{R}_{oo} = \frac{1}{k_2^*} - \frac{e^{-u}}{k_1^*}$$

$$R_{12} = \frac{1}{b_2} + \frac{e^{u/2}}{k_2^*} \qquad \dot{R}_{12} = 1/k_2^*$$

$$R_{21} = \frac{e^{u/2}}{b_1^*} + \frac{e^{-u/2}}{k_1^*} \qquad \dot{R}_{21} = \frac{1}{b_1} - \frac{e^{-u}}{k_1^*}$$

$$R_{ee} = \frac{e^{u/2}}{b_1^*} + \frac{1}{b_2} \qquad \dot{R}_{ee} = \frac{1}{b_1^*}$$

Hence $R_{12}$ and $R_{ee}$ increase linearly with $e^{u/2}$, while $K$ increases linearly with $e^{-u/2}$. $R_{oo}$ and $R_{21}$ display an non-linear dependence on $e^{u/2}$. By similar argu-

TABLE III

EFFECTS OF AN APPLIED VOLTAGE ON THE KINETIC PARAMETERS OF A CARRIER SYSTEM ON THE MODEL OF FIG. 2, WHERE ONLY ONE FORM OF ES EXISTS

This table records the results for all of the three subcases considered in the text. Where the result for all three subcases is the same only a single entry is made. Where the results differ, they are recorded at each row/column intersection in the order: side 1 only affected by field, side 2 only affected, both sides equally affected. The minus sign in brackets after linear means that the linear dependence is seen when the data are plotted against $e^{-u/2}$, rather than against $e^{u/2}$.

| Case (see Table II) | $K$ | $R_{oo}$ | $R_{12}$ | $R_{21}$ | $R_{ee}$ |
|---|---|---|---|---|---|
| I | linear (—), linear, non-linear | non-linear | linear, non-linear, non-linear | non-linear, linear (—), non-linear | linear, linear(—), non-linear |
| II | linear (—), linear, non-linear | Invariant | Invariant, linear (—), linear (—) | linear, Invariant, linear (—) | linear, linear (—), non-linear |
| III | Invariant | non-linear | linear | linear (—) | Invariant |
| IV | linear (—), linear, invariant | non-linear | linear, non-linear, non-linear | non-linear, linear (—), non-linear | linear (—), linear (—), non-linear |

ments, one completes Table III for the remaining cases, where the electric field affects only the on and off constants at side 1. The similar results for side 2 only being affected are also collected in the table. For the intermediate case where the on and off reactions at each side of the membrane extend into the middle of the field, the rate constants for Cases I through III are chosen from relations such as $k_1 = k_1^* e^{u/2}$; $k_2 = k_2^* e^{-u/2}$; $f_1 = f_1^* e^{u/4}$; $f_2 = f_2^* e^{-u/4}$; $b_1 = b_1^* e^{-u/4}$; $b_2 = b_2^* e^{u/4}$. For Case IV, the $f$ and $b$ relations contain terms in $u/2$. The relevant results are simply derived and are collected in Table III.

*Analysis of the effects of an applied field*

To use the theory developed in this paper, one must measure the relevant parameters $K$, $R_{12}$, $R_{21}$, $R_{ee}$ and $R_{oo}$ by the methods described in ref. 1 (these methods will involve the determination of velocities and half-saturation concentrations for transport), as a function of applied voltage. One then determines first which of the five parameters is invariant with the applied voltage. If this is $R_{oo}$, then on any model Case II holds, the free carrier is uncharged and the carrier-substrate complex is charged. If $R_{ee}$ is invariant, then whatever the model Case III applies and the free carrier and the substrate are oppositely and equally charged, so that the complex is uncharged. In addition, from the slope of the plot of $R_{12}$ or $R_{21}$ one can find the value of $k_1$ and $k_2$, whatever model or subcase is appropriate. If $R_{12}$, $R_{21}$ or $K$ are invariant with voltage, while $R_{ee}$ varies, it would appear that the model that best describes the system is that of Fig. 2, (Case II, III or IV, respectively). If $R_{12}$ or $R_{21}$ are invariant while $R_{ee}$ is also invariant, Case III is indicated and it is possible to argue in favour of a particular placing of the transition state for the $E_1 \rightleftharpoons E_2$ transformation, with regard to the position of the electric field.

One might then attempt the far more difficult task of attempting to use the distinction between a linear and a non-linear dependence of a particular parameter on the applied voltage. It will be heuristically useful if one finds, experimentally, a non-linear dependence but if a linear dependence is found it can always be argued that the range of voltages studied is as yet too low, and that the dependence will become non-linear at a more extreme voltage. One is, however, entitled to argue from the finding that $R_{oo}$ is invariant (Case II) while $R_{12}$ and $R_{21}$ behave non-linearly that the model of Fig. 1 is to be preferred over that of Fig. 2. One would thus have succeeded in distinguishing between these two models by using only steady-state measurements. The situation of $R_{ee}$ being invariant offers no such distinction.

If no parameter is invariant and all show a non-linear dependence on the applied voltage one can decide between Case I or Case IV according as to whether the substrate is uncharged or charged, respectively.

## Conclusions

The present note provides the theory by which the variation or lack of variation with the voltage applied across the membrane of the steady-state kinetic parameters of a carrier system might be interpreted in terms of the properties of the carrier. In two favourable circumstances, where the parameter $R_{oo}$ or the parameter $R_{ee}$ is invariant with voltage, unequivocal statements can be made about the charge on the free carrier. (It is uncharged in the former case and charged oppositely to the substrate in the latter). In certain special cases of invariant parameters additional statements can be made. In one special case, where the parameter $R_{oo}$ does not vary with the applied voltage, whereas the parameters $R_{12}$ and $R_{21}$ vary non-linearly with voltage, one can even argue that one has succeeded in identifying the isomerisation of the carrier-substrate complex, which up to now has not been possible using steady-state measurements. There still is no general procedure for making this distinction using steady-state data, however, and success with the particular special case discussed here need not require that in general one should use the conventional model of Fig. 1 in preference to the "necessarily simplified" model of Fig. 2.

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