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TESTING AND CHARACTERIZING THE SIMPLE PORE

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SUMMARY

1. We define a simple (or singly-occupied) pore and show that any pore which is fully occupied by a single substrate particle behaves as a simple pore at the level of steady-state analysis.

2. The analysis is independent of assumptions as to (a) symmetry, (b) equilibrium, (c) the distribution of electrical potential in the membrane, and (d) the presence or absence of unstirred layers.

3. We suggest simple experimental procedures involving only one substrate, the results of which can be used to test for and characterize the simple pore. For ions, only electrical measurements are needed.

INTRODUCTION

Karl Popper [1] writes that "the method of science is the method of bold conjectures and ingenious and severe attempts to refute them". But before one moves to the bold conjectures, one must first test severely, the most simple hypotheses. In this paper, we present tests for perhaps the most elementary of all transport models: the simple (or singly-occupied) pore. For those transport systems which pass the tests, we show how one can interpret the experimental measurements in terms of the minimum set of kinetic parameters. In the following paper [2], we deal with the simple carrier.

By a pore we mean a continuous passage through the membrane, open at both ends and (when unoccupied) accessible simultaneously to substrate at both sides of the membrane. The pore may or may not be lined with specific binding sites. This definition includes the proposed equivalent pore of Solomon [3], the long pore [4, 5], and the antibiotic pores [6]. By a simple pore we mean a pore which can be occupied by only one molecule or ion of substrate at any one time. The simple pore will include not only the one-site pore such as Hille [7] has proposed for the selectivity filter of the nerve Na^+ channel but also the multiple-site but nonetheless singly-occupied pore considered by Läuger [8] for such a channel as that formed by gramicidin, where it is thought that binding of a single cation shuts out binding of additional cations due to electrostatic repulsion. We use the term "simple" in the sense that the model requires the minimal number of occupancy states: a given pore is either empty or fully occupied so that partial occupancies need not be considered.

The power of a rejection criterion lies in its ability to cover as many particular forms of a hypothesis as possible. For this reason we have formulated some criteria which make no assumptions as to (1) symmetry, (2) equilibrium, (3) the distribution of electrical potential in the membrane, or (4) the presence or absence of unstirred layers. In general, it is not possible to predict how changes in transmembrane electrical potential will affect the individual rate constants of the model. For this reason we consider only experimental situations where the transmembrane voltage is fixed and where the ionic strength is constant and, for the case of ionic permeants, sufficiently high to make the electrical field in the unstirred layers negligible. Under these conditions we assume that the rate constants do not vary appreciably and do not depend upon the concentration of permeant. We also assume that the individual pores are embedded in the membrane and do not interact with one another.

PROCEDURE AND DISCUSSION

Steady-state solutions for the simple pore

We consider in Figs 1 and 2 two of the many possible forms of the simple pore. In each figure the pictorial representation of the model is on the left, its formal representation on the right. Symbols are as defined in Appendix. In a previous paper [9], we have solved both forms for the special case of zero transmembrane voltage. For the one-site pore of Fig. 1 the unidirectional flux of permeant from Side 1 to Side 2 of the membrane is

$$v_{1\to 2} = \frac{b_2 f_1 n S_1}{b_1 + b_2 + f_1 S_1 + f_2 S_2} \tag{1}$$

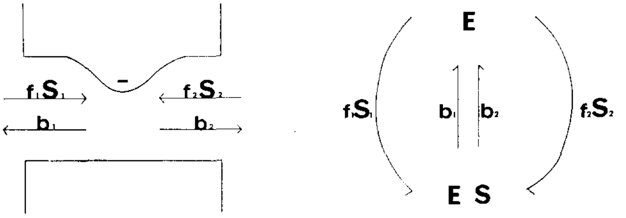

Fig. 1. The one-site pore.

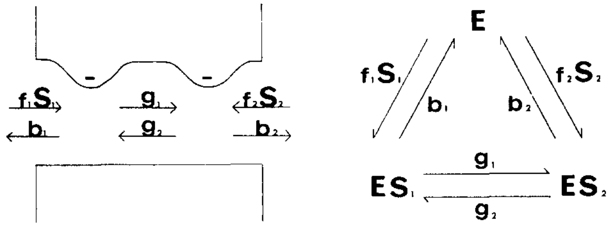

Fig. 2. The two-site pore with single occupancy only.

where S_1 and S_2 are the aqueous concentrations of permeant in the immediate vicinity of the pore at Sides 1 and 2, respectively. To take into account the effect of transmembrane voltage, one uses microscopic reversibility (as given by the Principle of Detailed Balance [10]) to obtain:

$$b_1 f_2 e^u = b_2 f_1 \tag{2}$$

where u is the reduced transmembrane potential difference $(zF/N_A kT)(\psi_1 - \psi_2)$. Using this relationship in Eqn 1 gives

$$v_{1\to 2} = \frac{Q_{21} S_1}{Q_{12}Q_{21} + Q_{21} R_{12} S_1 + Q_{12} R_{21} S_2} \tag{3}$$

where Q_{12}, Q_{21}, R_{12}, and R_{21} are as given in the appropriate column of Table I. The unidirectional flux from Side 2 to Side 1 is obtained by simply interchanging the subscripts 1 and 2 in Eqn 3.

For the two-site pore with single occupancy only (Fig. 2) the solution is

$$v_{1\to 2} = \frac{b_2 f_1 g_1 n S_1}{b_1 b_2 + b_1 g_2 + b_2 g_1 + f_1(b_2 + g_1 + g_2)S_1 + f_2(b_1 + g_1 + g_2)S_2} \tag{4}$$

and the microscopic reversibility condition gives

$$b_1 f_2 g_2 e^u = b_2 f_1 g_1 \tag{5}$$

Using this relationship in Eqn 4 gives precisely the same equation as Eqn 3, but with the symbols Q_{12}, Q_{21}, R_{12}, and R_{21} now having the meaning given in the last column of Table I.

TABLE I

STEADY-STATE SOLUTIONS FOR ONE- AND TWO-SITE FORMS OF SIMPLE PORE

$$v_{1\to 2} = \frac{Q_{21} S_1}{Q_{12}Q_{21} + Q_{21} R_{12} S_1 + Q_{12} R_{21} S_2}$$

where $Q_{21} = Q_{12} e^u$

	One-site pore	Two-site pore
$nR_{12} =$	$\frac{1}{b_2}$	$\frac{1}{b_2} + \frac{1}{g_1}\left(\frac{b_2+g_2}{b_2}\right)$
$nR_{21} =$	$\frac{1}{b_1}$	$\frac{1}{b_1} + \frac{1}{g_2}\left(\frac{b_1+g_1}{b_1}\right)$
$nQ_{12} =$	$\frac{b_1}{f_1}\left(\frac{1}{b_1} + \frac{1}{b_2}\right)$	$\frac{b_1}{f_1}\left[\frac{1}{b_1} + \frac{1}{g_1}\left(\frac{b_2+g_2}{b_2}\right)\right]$
$nQ_{21} =$	$\frac{b_2}{f_2}\left(\frac{1}{b_1} + \frac{1}{b_2}\right)$	$\frac{b_2}{f_2}\left[\frac{1}{b_2} + \frac{1}{g_2}\left(\frac{b_1+g_1}{b_1}\right)\right]$
Constraint:	$b_1 f_2 e^u = b_2 f_1$	$b_1 f_2 g_2 e^u = b_2 f_1 g_1$

Since from steady-state measurements one can obtain only Q_{12}, Q_{21}, R_{12}, and R_{21}, the important result which follows is that such measurements cannot distinguish between the two forms (Figs 1 and 2) of the simple pore. Indeed, this is only an extension to transport systems of the well-known result in enzyme kinetics that the presence of intermediate complexes $(ES)_1$, $(ES)_2$, $(ES)_3$, . . . , $(ES)_N$ cannot be detected by steady-state methods alone, without simplifying assumptions [11]. Thus the general result here is that any pore which can be occupied by only one substrate particle at a time will, no matter how many binding sites it possesses in a row going through the membrane, behave exactly like the one-site pore. This means, for example, that it is not possible on the basis of steady-state measurements to determine for the two-site pore of Fig. 2 whether movement within the pore (g_1 or g_2) is faster or slower than egress (b_1 or b_2). Läuger [8] has shown that by making certain assumptions about the voltage distribution across the pore and about the number of sites, it is possible to obtain such information. This is a very reasonable approach to follow when working with a system of known structure, but of course it is not applicable in general and certainly not when one is working with a transport system of unknown structure.

Description of the procedures

The rejection criteria for and the characterization of the simple pore will be in terms of observable parameters obtained from the following procedures, all of which are performed at constant transmembrane potential and ionic strength. In addition, for ion transport this ionic strength should be sufficiently high to make the electric field in the unstirred layers (if present) negligible.

(*i*) *Zero-trans procedures.* Here the concentration of substrate in the solution bathing the *trans* face of the membrane is kept at zero while that in the *cis* solution is varied. The net movement of substrate can be measured either chemically or electrically. There are two zero-*trans* procedures, according to whether Side 1 or Side 2 of the membrane is taken as the *trans* face.

(*ii*) *Cis = trans procedure.* Here the *cis* and *trans* bathing solutions are identical. At a nonzero transmembrane voltage, there will be a net flow of substrate, which can be measured either chemically or electrically. At zero transmembrane voltage there is, of course, no net flow. But one can characterize the system at zero voltage by the limiting ohmic conductance λ_0, defined as the limit of the ratio of current to voltage as the voltage approaches zero.

(*iii*) *Equilibrium-exchange procedure.* In this procedure the *cis* and *trans* bathing solutions are arranged to be at electrochemical equilibrium, that is, so that $S_1/S_2 = e^{-u}$. Since under this condition there is no net flow of substrate, one measures only the unidirectional flux, using tracers.

Analysis of the simple pore in the absence of unstirred layers

It is possible, as we show in a later section, to completely characterize the simple pore even if unstirred layers are present. Nevertheless, in order to introduce the concepts involved, it is useful to consider first the simpler situation of a perfectly stirred system. In this case S_1 and S_2, the substrate concentrations in the immediate vicinity of the pore, are also the concentrations in the bulk solutions. We now consider in turn the three experimental procedures defined in the preceding section.

(*i*) *Zero-trans procedures.* For the case when $S_2 = 0$, Eqn 3 reduces to

$$v^{zt}_{1\to2} = \frac{S_1}{Q_{12}+R_{12}S_1} \tag{6}$$

This is a simple Michaelis–Menten form with maximal velocity $V^{zt}_{1\to2} = 1/R_{12}$ and half-saturation concentration $K^{zt}_{1\to2} = Q_{12}/R_{12}$. For the case when $S_1 = 0$ similar results are obtained by interchanging subscripts 1 and 2. These and subsequent results are listed in Table II.

TABLE II

THE SIMPLE PORE: INTERPRETATION OF EXPERIMENTAL DATA IN TERMS OF BASIC PARAMETERS

Procedure		
Net flows★		
Zero-*trans*	$V^{zt}_{1\to2} = \frac{1}{R_{12}}$	$K^{zt}_{1\to2} = \frac{Q_{12}}{R_{12}}$
	$V^{zt}_{2\to1} = \frac{1}{R_{21}}$	$K^{zt}_{2\to1} = \frac{Q_{21}}{R_{21}}$
Cis=trans	$V^{c=t} = \frac{e^u-1}{R_{12}e^u+R_{21}}$	$K^{c=t} = \frac{Q_{12}e^{u/2}}{R_{12}e^{u/2}+R_{21}e^{-u/2}}$
	$= \frac{2\sinh(u/2)}{R_{12}e^{u/2}+R_{21}e^{-u/2}}$	$= \frac{Q_{21}e^{-u/2}}{R_{12}e^{u/2}+R_{21}e^{-u/2}}$
Cis=trans when $u \to 0$	$\Lambda_0 = \frac{z^2F^2}{N_A kT}\cdot\frac{1}{R_{12}+R_{21}}$	$K^{\lambda_0} = \frac{Q_0}{R_{12}+R_{21}}$
Unidirectional flows		
Equilibrium–exchange	$V^{ee} = V^{ee}_{1\to2} = V^{ee}_{2\to1}$	$K^{ee}_1 = \frac{Q_{12}}{R_{12}+R_{21}}$
	$= \frac{1}{R_{12}+R_{21}}$	$K^{ee}_2 = \frac{Q_{21}}{R_{12}+R_{21}}$

★ To convert net chemical flows V to electrical currents J multiply by zF.

(*ii*) *Cis = trans procedure*. Here $S_1 = S_2 = S$, so that from the difference between Eqn 3 and its analogue the net flux is

$$NET^{c=t}_{1\to2} = -NET^{c=t}_{2\to1} = v^{c=t}_{1\to2} - v^{c=t}_{2\to1} = \frac{(Q_{21}-Q_{12})S}{Q_{12}Q_{21}+(Q_{21}R_{12}+Q_{12}R_{21})S} \tag{7}$$

Since (see Table I) $Q_{21} = e^uQ_{12}$,

$$NET^{c=t}_{1\to2} = \frac{(e^u-1)S}{e^uQ_{12}+(e^uR_{12}+R_{21})S} \tag{8}$$

This is, once again, a simple Michaelis–Menten form with the maximal velocity and half-saturation concentration as given in Table II. The limiting ohmic conductance is

$$\lambda_0 = \lim_{(\psi_1-\psi_2)\to 0} \frac{zFNET_{1\to 2}^{c=t}}{(\psi_1-\psi_2)} = \frac{z^2F^2}{N_A kT} \cdot \lim_{u\to 0} \frac{NET_{1\to 2}^{c=t}}{u} = \frac{z^2F^2}{N_A kT} \cdot \frac{S}{Q_0+(R_{12}+R_{21})S} \tag{9}$$

The maximal limiting ohmic conductance Λ_0 and the half-saturation concentration K^{λ_0} are then as listed in Table II.

(*iii*) *Equilibrium-exchange procedure.* Here $S_1 = S_2 e^{-u}$. Since $Q_{21} = e^u Q_{12}$, Eqn 3 reduces to

$$v^{ee} = \frac{S_1}{Q_{12}+(R_{12}+R_{21})S_1} = \frac{S_2}{Q_{21}+(R_{12}+R_{21})S_2} \tag{10}$$

The maximal velocity for these Michaelis–Menten forms is $V^{ee} = 1/(R_{12}+R_{21})$. The half-saturation concentration can be defined either with reference to Solution 1 as $K_1{}^{ee} = Q_{12}/(R_{12}+R_{21})$ or with reference to Solution 2 as $K_2{}^{ee} = Q_{21}/(R_{12}+R_{21})$. Of course, $K_1{}^{ee} = e^{-u} K_2{}^{ee}$.

From the relations listed in Table II and the fact that $Q_{21} = e^u Q_{12}$ one can derive rejection criteria for the simple pore in the absence of unstirred layers. Some of the most convenient of these criteria are collected in Table III. These rejection criteria involve measurable parameters only. If the relation between the measured parameters is not that given by any one of these rejection criteria, then one is forced to conclude that the transport system in question is not a simple pore. Since each criterion must be true at every transmembrane voltage if the system is a simple pore, the number of possible tests far exceeds the number of rejection criteria. However, in a real system where one cannot assume a priori perfect mixing, some but not all of these criteria are applicable. We proceed to consider the problem of unstirred layers.

Analysis of the simple pore in the presence of unstirred layers

When unstirred layers are present, the concentrations S_1 and S_2 in the immediate vicinity of the pore may not be equal to the concentrations $S_1{}^B$ and $S_2{}^B$, respectively, in the bulk solutions (see Fig. 3). We can define for the unstirred layers, which may have any size and shape, effective permeability coefficients P_1 and P_2, respectively.

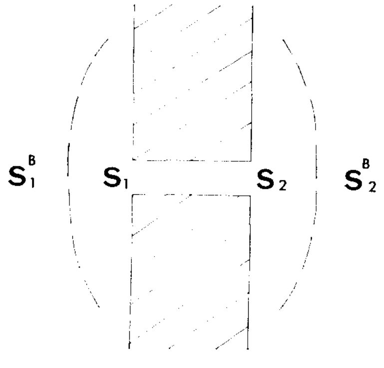

Fig. 3. The simple pore in the presence of unstirred layers.

TABLE III

SOME REJECTION CRITERIA FOR THE SIMPLE PORE★

I. Using electrical or chemical★★ measurements

A. For $u \neq 0$

$$* \quad \frac{\dfrac{1}{J^{c=t}} + \dfrac{1}{J^{zt}_{2\to1}}}{\dfrac{1}{J^{c=t}} - \dfrac{1}{J^{zt}_{1\to2}}} = e^{u},$$

which implies that

$$* \quad J^{zt}_{1\to2} \geqq \frac{J^{c=t}}{1-e^{-u}} \quad \text{and} \quad J^{zt}_{2\to1} \geqq \frac{J^{c=t}}{e^{u}-1}$$

$$\frac{1}{K^{c=t}} = \frac{1}{K^{zt}_{1\to2}} + \frac{1}{K^{zt}_{2\to1}},$$

which implies that

$$K^{zt}_{1\to2},\, K^{zt}_{2\to1} \geqq K^{c=t}$$

B. For $u = 0$

$$* \quad \frac{1}{\Lambda_0} = \frac{N_A kT}{zF}\left[\frac{1}{J^{zt}_{1\to2}} + \frac{1}{J^{zt}_{2\to1}}\right] \quad \text{and} \quad \frac{1}{K^{\lambda_0}} = \frac{1}{K^{zt}_{1\to2}} + \frac{1}{K^{zt}_{2\to1}},$$

together with implied inequalities as above.

II. Using chemical measurements only

For all values of u:

$$* \quad \frac{1}{V^{ee}} = \frac{1}{V^{zt}_{1\to2}} + \frac{1}{V^{zt}_{2\to1}}$$

which implies that

$$* \quad V^{zt}_{1\to2},\, V^{zt}_{2\to1} \geqq V^{ee}$$

$$\frac{K^{ee}_1}{K^{zt}_{1\to2}} + \frac{K^{ee}_2}{K^{zt}_{2\to1}} = 1,$$

which implies that

$$K^{zt}_{1\to2} \geqq K^{ee}_1 \quad \text{and} \quad K^{zt}_{2\to1} \geqq K^{ee}_2$$

III. Using both electrical and chemical measurements

For u = 0:

$$* \quad \Lambda_0 = \frac{z^2F^2}{N_A kT} V^{ee}$$

$$K^{\lambda_0} = K^{ee}$$

★ Rejection criteria on lines marked with an asterisk are valid even in the presence of unstirred layers.

★★ For chemical measurements substitute zFV for J.

Since the net flow of substrate (or of tracer in the equilibrium–exchange procedure) in the steady-state is the same (1) from bulk solution to membrane as (2) through the membrane and as (3) from membrane to bulk solution on the other side, it is possible to obtain S_1 and S_2 in terms of $S_1{}^{\mathrm{B}}$, $S_2{}^{\mathrm{B}}$, P_1, and P_2.

(*i*) *Zero-trans procedures.* For the case when $S_2{}^{\mathrm{B}} = 0$,

$$S_1 = S_1^{\mathrm{B}} - NET_{1\to2}^{\mathrm{zt}}/P_1 \tag{11}$$

and

$$S_2 = NET_{1\to2}^{\mathrm{zt}}/P_2 \tag{12}$$

Substituting for S_1 and S_2 in Eqn 3 and its analogue and using the relation $Q_{21} = \mathrm{e}^{u}Q_{12}$ gives

$$NET_{1\to2}^{\mathrm{zt}} = \frac{S_1^{\mathrm{B}} - \left(\dfrac{1}{P_1} + \dfrac{\mathrm{e}^{-u}}{P_2}\right) NET_{1\to2}^{\mathrm{zt}}}{Q_{12} + R_{12}S_1^{\mathrm{B}} + \left(\dfrac{\mathrm{e}^{-u}R_{21}}{P_2} - \dfrac{R_{12}}{P_1}\right) NET_{1\to2}^{\mathrm{zt}}} \tag{13}$$

This is not a Michaelis–Menten form (unless P_1/R_{21} happens to be equal to $\mathrm{e}^{u}P_2R_{12}$). Nevertheless, the maximum velocity can be seen to be

$$V_{1\to2}^{\mathrm{zt}} = \frac{1}{R_{12}} \tag{14}$$

which is precisely the result found in the absence of unstirred layers (see Table II). The analogous result is found for $V_{2\to1}^{\mathrm{zt}}$, for the case when $S_1{}^{\mathrm{B}} = 0$.

(*ii*) *Cis = trans procedure.* Here $S_1{}^{\mathrm{B}} = S_2{}^{\mathrm{B}} = S^{\mathrm{B}}$ so that

$$S_1 = S^{\mathrm{B}} - NET_{1\to2}^{\mathrm{c=t}}/P_1 \tag{15}$$

and

$$S_2 = S^{\mathrm{B}} + NET_{1\to2}^{\mathrm{c=t}}/P_2 \tag{16}$$

Substituting for S_1 and S_2 as above we obtain

$$NET_{1\to2}^{\mathrm{c=t}} = \frac{(1-\mathrm{e}^{-u})S^{\mathrm{B}} - \left(\dfrac{1}{P_1} + \dfrac{\mathrm{e}^{-u}}{P_2}\right) NET_{1\to2}^{\mathrm{c=t}}}{Q_{12} + (R_{12} + \mathrm{e}^{-u}R_{21})S^{\mathrm{B}} + \left(\dfrac{\mathrm{e}^{-u}R_{21}}{P_2} - \dfrac{R_{12}}{P_1}\right) NET_{1\to2}^{\mathrm{c=t}}} \tag{17}$$

Once again this is not a Michaelis–Menten form (unless $P_1R_{21} = \mathrm{e}^{u}P_2R_{12}$), but the maximum velocity is

$$V^{\mathrm{c=t}} = \frac{2\sinh(u/2)}{R_{12}\mathrm{e}^{u/2} + R_{21}\mathrm{e}^{-u/2}} \tag{18}$$

which is the same result found in the absence of unstirred layers (see Table II). The limiting ohmic conductance is

$$\lambda_0 = \frac{z^2F^2}{N_A kT} \cdot \frac{S^B}{Q_0 + \frac{1}{P_1} + \frac{1}{P_2} + (R_{12}+R_{21})S^B} \tag{19}$$

This is a Michaelis–Menten form with half-saturation concentration

$$K^{\lambda_0} = (Q_0 + P_1^{-1} + P_2^{-1})/(R_{12}+R_{21})$$

and maximal value

$$\Lambda_0 = \frac{z^2F^2}{N_A kT} \cdot \frac{1}{R_{12}+R_{21}} \tag{20}$$

The maximum limiting ohmic conductance is thus the same in the presence or absence of unstirred layers (see Table II).

(*iii*) *Equilibrium-exchange procedure*. Since the system here is in electrochemical equilibrium, there are no net flows of total (labeled+unlabeled) substrate. Therefore the total concentration at each membrane surface is the same as that in the adjacent bulk solution. However, since there is a net flow of labeled substrate (tracer), the concentration of tracer in the immediate vicinity of the pore will not be equal to that in the adjacent bulk solution. If we use starred symbols to represent tracer quantities and unstarred for total quantities, then for the case of $^*S_2{}^B = 0$ we have that

$$^*S_1 = {}^*S_1^B - \frac{^*NET_{1\to 2}^{ee}}{P_1} \tag{21}$$

$$^*S_2 = \frac{^*NET_{1\to 2}^{ee}}{P_2} \tag{22}$$

$$S_1 = S_1^B = e^{-u}S_2^B = e^{-u}S_2 \tag{23}$$

where $^*NET_{1\to 2}^{ee}$ is the net flow of tracer from Bulk Solution 1 to Bulk Solution 2. But the net flow of tracer between the bulk solutions equals the net flow of tracer through the pore, which in turn equals the total flow through the pore (given by Eqn 10 since the equalities in Eqn 23 for total concentrations exist) multiplied by the difference of the specific activities of tracer in the immediate vicinities of the pore at the two faces, i.e.

$$^*NET_{1\to 2}^{ee} = \frac{S_1}{Q_{12}+(R_{12}+R_{21})S_1}\left(\frac{^*S_1}{S_1} - \frac{^*S_2}{S_2}\right) \tag{24}$$

Using Eqns 21–23 in Eqn 24 and then collecting terms leads to

$$^*NET_{1\to 2}^{ee} = \frac{^*S_1^B}{Q_{12} + \frac{1}{P_1} + \frac{e^{-u}}{P_2} + (R_{12}+R_{21})S_1^B} \tag{25}$$

Since the total unidirectional flux v^{ee} from bulk solution to bulk solution is equal to $^*NET_{1\to 2}^{ee}$ divided by the specific activity $^*S_1{}^B/S_1{}^B$ in Bulk Solution 1, it follows immediately that

$$v^{ee} = \frac{S_1^B}{Q_{12} + \frac{1}{P_1} + \frac{e^{-u}}{P_2} + (R_{12}+R_{21})S_1^B} \tag{26}$$

This is a Michaelis–Menten form with half-saturation concentrations

$$K_1^{ee} = \frac{Q_{12} + \frac{1}{P_1} + \frac{e^{-u}}{P_2}}{R_{12}+R_{21}} \tag{27}$$

and $K_2^{ee} = e^u K_1^{ee}$ with reference to Bulk Solutions 1 and 2, respectively, and with a maximal velocity of

$$V^{ee} = \frac{1}{R_{12}+R_{21}} \tag{28}$$

This maximal velocity is precisely that found in the absence of unstirred layers.

We have, therefore, the very interesting result that all of the maximal velocities and also the maximal limiting ohmic conductance are unaffected by the presence of unstirred layers. This means that all of the rejection criteria involving only these maximal parameters are valid for both well-stirred and unstirred systems. We have indicated these invariant rejection criteria with an asterisk in Table III.

Characterization of the simple pore with or without unstirred layers

Since one now has rejection criteria for the simple pore even in the presence of unstirred layers, one can test experimentally the validity of the simple pore model for any given transport system. For a system which indeed behaves as a simple pore, a complete characterization of the system in terms of the basic measurable parameters R_{12}, R_{21}, Q_{12}, Q_{21}, P_1, and P_2 and in terms of relative values of the molecular rate constants, b_1, b_2, f_1, and f_2 of the one-site form of the simple pore can be obtained as follows.

All of the required information can be obtained from the two zero-*trans* procedures, which have the advantage of being able to be performed by either chemical or electrical means. The terms R_{12} and R_{21} are simply the reciprocals of the maximal velocities of the two zero-*trans* procedures (see Table II). The terms Q_{12}, Q_{21}, P_1, and P_2 are obtained by a closer examination of the results of these procedures, as we now show.

When the *trans* solution is Solution 2, a rearrangement of Eqn 13 gives

$$\frac{S_1^B}{NET_{1\to2}^{zt}} = Q_{12} + \frac{1}{P_1} + \frac{e^{-u}}{P_2} + R_{12}S_1^B + \left(\frac{e^{-u}R_{21}}{P_2} - \frac{R_{12}}{P_1}\right) NET_{1\to2}^{zt} \tag{29}$$

If for clarity we write $v = NET_{1\to2}^{zt}$ and $V = V_{1\to2}^{zt} = 1/R_{12}$, this equation can be expressed as

$$S_1^B\left(\frac{1}{v} - \frac{1}{V}\right) = Q_{12} + \frac{1}{P_1} + \frac{e^{-u}}{P_2} + \left(\frac{e^{-u}R_{21}}{P_2} - \frac{R_{12}}{P_1}\right) v \tag{30}$$

If one plots the left-hand side of Eqn 30 against v one should obtain a straight line of slope $m = (e^{-u}R_{21}P_2^{-1} - R_{12}P_1^{-1})$ and intercept on the ordinate axis of $(Q_{12}+P_1^{-1}+e^{-u}P_2^{-1})$.

The slope m contains the unknown parameters P_1 and P_2, the permeability coefficients of the unstirred layers. It is not to be expected that these will be affected by changes in transmembrane voltage, although R_{12} and R_{21} will in general be altered. Thus by measuring m, R_{12}, and R_{21} at many different voltages u, the constant coefficients $1/P_1$ and $1/P_2$ in the relation

$$m(u) = \frac{1}{P_2} e^{-u} R_{21}(u) - \frac{1}{P_1} R_{12}(u) \qquad (31)$$

can easily be obtained, together with estimates of error, from a least squares regression of $m(u)$ upon $e^{-u}R_{21}(u)$ and $R_{12}(u)$. If $1/P_1$ and $1/P_2$ are found to be statistically indistinguishable from zero, then of course no unstirred layer is detectable within the precision of the experiment.

Finally, P_1 and P_2 being known, Q_{12} and $Q_{21} = e^u Q_{12}$ at any transmembrane voltage can be found from the intercept on the ordinate axis in the plot of Eqn 30. A similar analysis of the results of the zero-*trans* procedure in the opposite direction will provide a useful check.

We have now shown how one can obtain R_{12}, R_{21}, Q_{12}, Q_{21}, P_1, and P_2. Knowing these, the molecular rate constants b_1, b_2, f_1, and f_2 for the one-site version of the simple pore can be obtained absolutely (if the concentration n of pores is known) or relatively from the simple relations given in Table IV. From the experimental results at different transmembrane voltages, one can find the voltage dependence of these molecular rate constants.

TABLE IV

RATE CONSTANTS FOR ONE-SITE VERSION OF SIMPLE PORE DERIVED FROM MEASURABLE PARAMETERS

$$b_1 = \frac{1}{nR_{21}}$$

$$b_2 = \frac{1}{nR_{12}}$$

$$f_1 = \frac{R_{12}+R_{21}}{nQ_{12}R_{21}}$$

$$f_2 = \frac{R_{12}+R_{21}}{nQ_{21}R_{12}}$$

Suggested steps for the testing and characterization of the simple pore

When approaching an unknown transport system suspected of being a simple pore, one might proceed as follows:

(1) Determine the maximal velocities of transport in the two zero-*trans* proce-

dures and in at least one of the *cis* = *trans* or equilibrium–exchange procedures, and apply the starred rejection criteria in Table III. For extra rigour, this might be done at many different transmembrane voltages. If the system fails any one of these criteria, it is not a simple pore and a more complex model must be considered. Otherwise, proceed to step (2).

(2) Determine the permeability coefficients of the unstirred layers using the two zero-*trans* procedures, as shown in the preceding section. (If unstirred layers are found to be insignificant, then the system can be further tested by applying the unstarred rejection criteria of Table III.)

(3) The transport system, having passed all of the tests, must be considered as a simple pore. This of course does not mean that it is indeed a simple pore, but only that one has no right to assume more complex models. Finally, using the methods of the preceding section, complete the steady-state characterization of the system by calculating relative or absolute values for the molecular rate constants of the one-site version of the simple pore model.

A major advantage of this approach is that all of the testing and characterization of the simple pore can, for ions, be accomplished using electrical methods only.

APPENDIX

List of symbols

b_1, b_2	effective interfacial rate constants for breakdown of ES to E and S
E	unoccupied pore
ES	pore occupied by substrate
f_1, f_2	effective interfacial rate constants for formation of ES from E and S
F	Faraday constant
g_1, g_2	rate constants for interconversion of ES_1 and ES_2 for two-site version of simple pore
j	net electrical current per unit area of membrane
J	maximum value of j
$\mathbf{k}$	Boltzman constant
K	that substrate concentration at which half of the maximum rate of transport occurs
K^{λ_0}	that substrate concentration in the *cis*=*trans* procedure at which λ_0 is half of Λ_0
n	total number of pores per unit area of membrane
N_A	Avogadro constant
NET	rate of net transport per unit area of membrane
P	effective permeability coefficient of unstirred layer on one side of membrane, per unit area of membrane
Q_{12}, Q_{21}	basic measurable membrane transport parameters
Q_0	value of Q_{12} and Q_{21} when $u = 0$
R_{12}, R_{21}	additional basic measurable membrane transport parameters
S	substrate concentration in immediate vicinity of pore
S^B	substrate concentration in bulk solution
T	absolute temperature

u	reduced transmembrane electrical potential difference, $(zF/N_AkT)(\psi_1-\psi_2)$
v	rate of unidirectional transport per unit area of membrane
V	maximum value of either *NET* or v
z	electrical valence of substrate
λ_0	limiting ohmic conductance per unit area of membrane as $u \rightarrow 0$ in *cis*⇌*trans* procedure
Λ_0	maximum value of λ_0
ψ	electrical potential

Except where explicitly specified otherwise in the above list, the superscript and subscript conventions are as follows:

Superscripts

c⇌t	*cis*⇌*trans* procedure
ee	equilibrium–exchange procedure
zt	zero-*trans* procedure

Subscripts

1	value at Side 1
2	value at Side 2
1 → 2	value in an experiment in which transport is measured from Side 1 to Side 2
2 → 1	value in an experiment in which transport is measured from Side 2 to Side 1

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