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# Quantitative relationships between the measurable transport resistances of membrane carriers: theory and experiment

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**A theoretical analysis is made of the possible quantitative relationships between the transport resistances that characterise membrane carrier systems. It is shown that there exist only five possible patterns in which to rank the four transport resistances. Symbolising these as $A$, $B$, $C$ and $D$, the five possible patterns are (i) $A=B=C=D$; (ii) $A=B \gg C, D$; (iii) $A=B \gg C=D$; (iv) $A=B=2C=2D$; (v) $A=2B=2C \gg D$. A survey of the available experimental data shows that pattern (ii) is the most prevalent, pattern (v) is often found and pattern (iii) has been identified. None of the ten transport systems so far analysed experimentally failed to fit one of the predicted patterns.**

## Introduction

For a number of years, Dr. W.R. Lieb and the author have been recommending the use of measurable transport resistances in characterising the kinetics of membrane transport [1–3]. These resistances are the reciprocals of the maximum velocity of transport measured under different, defined experimental situations. The present paper reports some newly-discovered inter-relationships between these measurable resistances. Only certain patterns in their quantitative ranking are predicted to occur. From these patterns, the rate-limiting steps in transport can readily be identified. The available data are in excellent agreement with the theoretical predictions.

## Definitions

Fig. 1 depicts the carrier model to be analysed. In Fig. 1a there is one form of the carrier-substrate complex, in Fig. 1b, two forms, and in Fig. 1c, three forms each of the free carrier and of the carrier-substrate complex [4]. The analysis to be given is quite general and does not require a distinction to be made between these cases (but see Deves and Krupka [5] for an experimental paradigm that enables a distinction to be made between 1a and 1b). Indeed, the analysis that follows is applicable also to those more complex carrier models where multiple intermediate carrier and carrier-substrate complexes can exist. Table I (modified from Ref. 4) defines the measurable membrane resistances $R_{12}$, $R_{21}$, $R_{ee}$, and $R_{oo}$ in terms of the rate constants of Fig. 1.

Experimentally, $R_{12}$ is obtained as the reciprocal of the maximum velocity of the zero *trans* or infinite *cis* transport in the 1-to-2 direction, $R_{12}$ similarly for the 2-to-1 direction, $R_{ee}$ as the reciprocal of the maximum velocity of the equilibrium exchange or the infinite *trans* exchange in either direction. $R_{oo}$, is obtained from the identity

$$R_{oo} = R_{12} + R_{21} - R_{ee}$$

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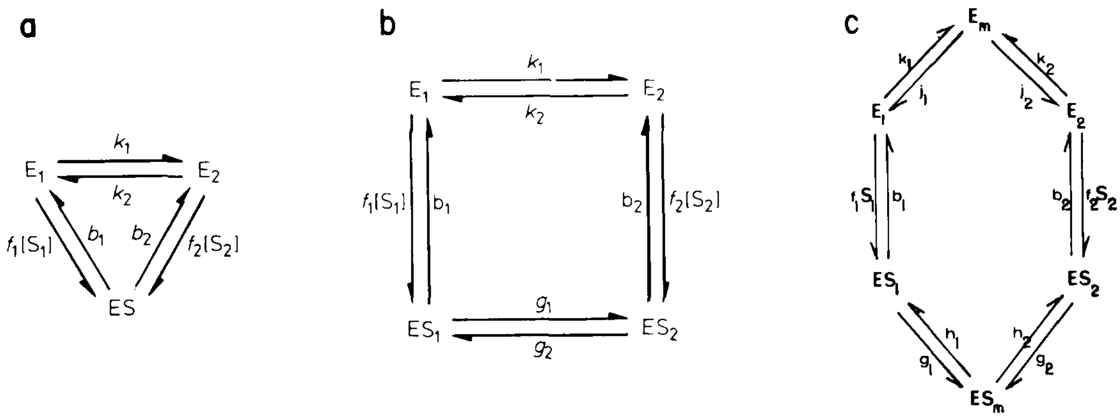


Fig. 1. Three forms of the simple carrier model. (a), the form with one state of the carrier-substrate complex; (b), the form with two states of this complex; (c) the form with three states of the carrier and three states of the carrier-substrate complex. E symbolises the carrier, S the substrate. The rate constants are as depicted on the figure. Subscripts 1 and 2 refer to steps present in, or originating at, sides 1 and 2 of the membrane respectively.

Each measurable resistance parameter is composed of two sets of combinations of rate constants. One set relates to the overall transport resistance in the 1-to-2 direction whether of loaded or unloaded carrier, the other set relates to the overall resistance in the 2-to-1 direction, again of

TABLE I

STEADY-STATE SOLUTIONS FOR THREE FORMS OF THE SIMPLE CARRIER

$v_{1\to2}$ is the unidirectional flux from solution 1 to solution 2; the reverse flux $v_{2\to1}$ can be obtained by interchanging subscripts 1 and 2 in the equation for $v_{1\to2}$. $S_1$ and $S_2$ are the permeant concentrations in solutions 1 and 2, while $n$ is the total concentration of carriers. The derived equation is independent of the form used when expressed in terms of the observable parameters $K$, $R_{12}$, $R_{21}$, $R_{ee}$, and $R_{oo}=R_{12}+R_{21}-R_{ee}$.

$$v_{1\to2}=\frac{(K+S_2)S_1}{K^2R_{oo}+KR_{12}S_1+KR_{21}S_2+R_{ee}S_1S_2}$$

| | Scheme of Fig. 1a | Scheme of Fig. 1b | Scheme of Fig. 1c |
|---|---|---|---|
| $nR_{12}=$ | $\frac{1}{b_2}+\frac{1}{k_2}$ | $\frac{1}{b_2}+\frac{1}{k_2}+\frac{1}{g_1}+\frac{g_2}{b_2g_1}$ | $\frac{1}{b_2}+\frac{1}{j_1}+\frac{1}{h_2}\left(\frac{b_2+g_2}{b_2}\right)+\frac{1}{k_2}\left(\frac{j_1+j_2}{j_1}\right)+\frac{1}{g_1}\left[1+\frac{h_1}{h_2}\left(\frac{b_2+g_2}{b_2}\right)\right]$ |
| $nR_{21}=$ | $\frac{1}{b_1}+\frac{1}{k_1}$ | $\frac{1}{b_1}+\frac{1}{k_1}+\frac{1}{g_2}+\frac{g_1}{b_1g_2}$ | $\frac{1}{b_1}+\frac{1}{j_2}+\frac{1}{h_1}\left(\frac{b_1+g_1}{b_1}\right)+\frac{1}{k_1}\left(\frac{j_1+j_2}{j_2}\right)+\frac{1}{g_2}\left[1+\frac{h_2}{h_1}\left(\frac{b_1+g_1}{b_1}\right)\right]$ |
| $nR_{ee}=$ | $\frac{1}{b_1}+\frac{1}{b_2}$ | $\frac{1}{b_1}+\frac{1}{b_2}+\frac{1}{g_1}+\frac{1}{g_2}+\frac{g_1}{b_1g_2}+\frac{g_2}{b_2g_1}$ | $\frac{1}{b_1}+\frac{1}{b_2}+\frac{1}{h_1}\left(\frac{b_1+g_1}{b_1}\right)+\frac{1}{h_2}\left(\frac{b_2+g_2}{b_2}\right)+\frac{1}{g_1}\left[1+\frac{h_1}{h_2}\left(\frac{b_2+g_2}{b_2}\right)\right]+\frac{1}{g_2}[1+\frac{h_2}{h_1}(\frac{b_1+g_1}{b_1})]$ |
| $nR_{oo}=$ | $\frac{1}{k_1}+\frac{1}{k_2}$ | $\frac{1}{k_1}+\frac{1}{k_2}$ | $\frac{1}{k_1}+\frac{1}{k_2}+\frac{1}{j_1}+\frac{1}{j_2}+\frac{j_1}{j_2k_1}+\frac{j_2}{j_1k_2}$ |
| $K=$ | $\frac{k_1}{f_1}+\frac{k_2}{f_2}$ | $\frac{k_1}{f_1}+\frac{k_2}{f_2}+\frac{k_1k_1}{f_1g_1}$ | $\frac{k_1}{f_1}\left(\frac{b_1+g_1}{g_1}\right)\left(\frac{j_2}{j_1+j_2}\right)+\frac{k_2}{f_2}\left(\frac{b_2+g_2}{g_2}\right)\left(\frac{j_1}{j_1+j_2}\right)$ |
| Constraints: | $b_1f_2k_1=b_1f_1k_2$ | $b_1f_2g_2k_1=b_2f_1g_1k_2$ | $b_1f_2g_2h_1j_2k_1=b_2f_1g_1h_2j_1k_2$ |

loaded or unloaded carrier. The overall resistance can be divided up among many possible intermediate transport steps, but it is always describable as a single lumped resistance term, readily derivable by the analysis of networks of resistance (see Ref. 3). All transport models of the general form of Fig. 1, where one substrate molecule combines with one transporter, can thus be reduced to the form of Fig. 1a, where each rate constant on that figure $b_1$, $b_2$, $k_1$, and $k_2$ is the reciprocal of the equivalent lumped resistance of the more complex path that they symbolise. For instance, rate constant $b_1$ in Fig. 1a is equivalent to the composite $b_1 g_2/(g_1 + g_2)$ in Fig. 1b, while $k_2$ in Fig. 1a is equivalent to the composite $k_2 j_1/(j_1 + j_2 + k_2)$ in Fig. 1c. One can thus discuss the general problem of the decomposition of the measurable transport parameters $R$ in terms of the simple model of Fig. 1a, realising that all carrier models in which one substrate molecule only can combine with one transporter can be brought into this form.

Each lumped resistance term describing an overall transport step, equivalent to the reciprocal of the rate constants $k$ and $b$ of Fig. 1a, appears in two of the measurable resistance parameters in $R$, each of which is the sum of two such lumped resistances termed the 'conjugate lumped resistances' of that measurable resistance parameter. Certain pairs of lumped resistances are never found combined in any measurable resistance parameter. I term these 'forbidden' conjugates. They are the pairs describing two overall transport paths in the same direction. All other conjugate pairs are 'allowable'.

When general statements about the measurable membrane resistance parameters are made, the symbols $A$, $B$, $C$, and $D$ are used, where $A$ can be any one of the four resistances, $B$ another, $C$ the third, and $D$ the fourth. Similarly, the four lumped resistances are in general statements referred to as $a$, $b$, $y$, and $z$, where $A = y + z$; $B = a + y$; $C = b + z$; and $D = a + b$. The forbidden conjugates are, therefore, $a$ and $z$, $b$ and $y$.

## Theory

The premise on which this analysis is based is the following: The lumped resistances of the transport model of Fig. 1 will be of very different sizes in general. In any measurable resistance parameter, one member of the conjugate pair of lumped resistances that comprises this measurable resistance will often be large and hence will dominate the overall resistance. We explore, in turn, the consequences (for all four measurable resistance parameters) of the various cases where one lumped resistance is outstandingly high, where two are high, where three are high, and finally, where all four are equally high (or low). (Although no strictly quantitative statements will be made, the words 'high', 'large', or 'greater' will refer to factors greater than about four, when the 'smaller' term will be negligible, being less than 20% of the overall value.)

*Case (i)*

One lumped resistance is very much larger than all the others. The two measurable resistances that contain this lumped resistance will be high and almost equally so. The pattern is then $A=B \gg C, D$. (Proof: put $y \gg z, a, b$ in the definitions of $A$, $B$, $C$, and $D$. Then $A=B \gg C, D$.)

*Subcase (a).* The next largest lumped resistance is the forbidden conjugate of the largest lumped resistance and is larger than the remaining two lumped resistances. The pattern is then $A=B \gg C=D$. (Proof: put $b$, the forbidden conjugate of $y$, as $\gg a$ or $z$ i.e. $y \gg b \gg a, z$. Then $C=D$ and hence $A=B \gg C=D$.) The same result will be found if, say, $y$ is large and if, by coincidence, $a$ and $z$ are equal and larger than $b$, the forbidden conjugate of the largest term, $y$.

*Subcase (b).* The next larger lumped resistance is an allowed conjugate of the largest. The pattern is then: $A=B \gg C > D$, where $C$ contains the second larger lumped resistance, taken arbitrarily as $z$.

*Case (ii)*

Two transport lumped resistances are very much larger than the other two, and are comparable in magnitude. (If they are not comparable, case (i) applies.)

*Subcase (a).* The two large rate constants are members of a forbidden pair. The pattern is then $A=B=C=D$. (Proof: the large lumped resistances never appear together in the same measurable transport resistance. They each appear, therefore, separately in two measurable resistances, and thus all four measurable resistances are approximately equal.)

*Subcase (b).* The two largest lumped resistances are members of an allowed pair. The pattern is then $A=2B=2C \gg D$ where $A$ is that measurable membrane resistance in which the two largest lumped resistances appear together. (Proof: parameter $A$ contains both the largest lumped resistances and is hence doubly large. Each large lumped resistance appears in another measurable membrane resistance, $B$ or $C$, which has then only one dominant term. A fourth measurable resistance is devoid of the conjugate pair and is hence small.)

*Case (iii)*

Three lumped resistances are large and are of comparable magnitude. (If they are not comparable, cases (ii) or (i) apply.) The pattern is then $A=B=2C=2D$. (Proof: the three lumped resistances will always be found in two allowable pairs and an overlapping forbidden pair. Let $a$, $b$, and $y$ be these large lumped resistances. The pairs $a$ and $b$, and $a$ and $y$ are allowable, the pair $b$ and $y$ forbidden. Then the two measurable resistances that comprise the two allowable pairs will be doubly large, containing two equally dominating terms, the remaining two measurable resistances containing one dominating term only from the forbidden pair will be half this size.)

*Case (iv)*

No lumped resistance is particularly small or large. The pattern is then $A=B=C=D$.

This completes the catalogue of cases.

## Predictions and Interpretations

The theoretically allowable patterns for the measurable membrane resistances, and the interpretation of these patterns, are given below.

*Pattern I: $A=B=C=D$*

*Interpretation.* (a) No lumped resistance is particularly large or small or (b) two lumped resistances, being members of a forbidden pair, are both large. (In this latter case, the four measurable resistance parameters are likely to be found as pairs $A=B \geqq C=D$, but this situation may be found for the former case by coincidence.)

*Pattern II: $A=B \gg C, D$*

*Interpretation:* One lumped resistance is very much larger than all the others. It appears as the common term in the two measurable resistances that are dominatingly large.

*Pattern III: $A=B \gg C=D$*

*Interpretation.* This is a subcase of II. One lumped resistance is exceptionally large (and is the common term of the two large measurable resistances), its forbidden conjugate is the next largest lumped resistance and is far larger than the remaining two. (This pattern is also found if, by coincidence, the two lumped resistances that are allowable conjugates of the largest lumped resistance, are themselves large and nearly equal to one another.)

*Pattern IV: $A=B=2C=2D$*

*Interpretation.* Three lumped resistances are large and comparably so (that is, only one lumped resistance is negligible. The forbidden conjugate pair among the three appears separately in the two smaller measurable resistances, along with the single small lumped resistance.

*Pattern V: $A=2B=2C \gg D$*

*Interpretation.* The lumped resistances comprising the two members of an allowed pair are very large. The measurable resistance in which they both appear is doubly large, the measurable resistances in which they appear once only are half this big, while the fourth, in which they do not appear, is small.

This completes the list of permitted patterns. In particular, it should be noted that the following pattern should never be found: $A \gg 2B, 2C, 2D$. NO measurable single resistance term can ever, on the carrier model be bigger than twice the other three resistances. This conclusion follows because every lumped resistance appears in two measurable resistances.

## Experimental data

Table II lists all those cases in the literature where I have been able to find sufficient data to extract the four measurable membrane resistance parameters. There are 11 such studies. Six (or seven) of these follow prediction II in the list above, four (or three) of them follow prediction V, one follows prediction III (a subcase of II). All patterns found are readily classifiable among the predicted patterns. Where, in addition, in any study the lumped resistances appropriate to the carrier model have been obtained, they confirm the interpretations proposed for the patterns of resistances (last column of Table II).

## Discussion and Conclusions

The predictions of the analysis seem, thus far, to be borne out by the available experimental data. This does not mean that future studies will necessarily be in accordance with the predictions, but it certainly seems worthwhile to extend further the testing of these relationships. Until discrepan-

TABLE II

EXPERIMENTAL DATA ON MEMBRANE RESISTANCES, AND THEIR INTERPRETATION [a]

| System | $R_{12}$ [a] | $R_{21}$ [a] | $R_{ee}$ | $R_{oo}$ | Prediction | Interpretation | Confirmation [b] |
|---|---|---|---|---|---|---|---|
| Human red blood cell | | | | | | | |
| Uridine (stored cells) [c] | $0.51 \pm 0.08$ | $1.89 \pm 0.14$ | $0.13 \pm 0.01$ | $2.26 \pm 0.16$ | II | $k_1$ small | Yes |
| Uridine (fresh cells) [d] | $0.74 \pm 0.08$ | $0.86 \pm 0.10$ | $0.22 \pm 0.03$ | $1.4 \pm 0.14$ | V | $k_1 \simeq k_2$, both small | Yes |
| Uridine (stored cells) [d] | $0.85 \pm 0.13$ | $2.8 \pm 0.3$ | $0.28 \pm 0.03$ | $3.4 \pm 0.34$ | IV | $k_1$ small | Yes |
| Glucose | $0.34 \pm 0.10$ | $1.96 \pm 0.16$ | $0.19 \pm 0.03$ | $2.11 \pm 0.19$ | II | $k_1$ small | – |
| Leucine (fresh cells) [f] | $1.49 \pm 0.03$ | $1.59 \pm 0.16$ | $0.76 \pm 0.08$ | $2.32 \pm 0.18$ | V | $k_1 \simeq k_2$, both small | Yes |
| Leucine (fresh cells) [g] | $1.30 \pm 0.07$ | $1.09 \pm 0.02$ | $0.62 \pm 0.02$ | $1.76 \pm 0.11$ | V | $k_1 \leqslant 1 k_2$, both small | Yes |
| Human lymphocytes | | | | | | | |
| 3-*O*-Me-glucose [h] | 0.26 | 0.93 | 0.23 | 0.97 | III | $k_1$ small, $b_2$ ($g_1$) next | – |
| Human fibroblasts Arginine [i] | $1.33 \pm 0.11$ | $0.67 \pm 0.21$ | $0.16 \pm 0.02$ | $1.84 \pm 0.24$ | II | $k_2$ small | Yes |
| HTC hepatoma cells Arginine [i] | $0.30 \pm 0.02$ | $0.50 \pm 0.14$ | $0.028 \pm 0.005$ | $0.78 \pm 0.14$ | V (or II) | $k_1 \leq k_2$, both small | Yes |
| Chlorella 6-Deoxyglucose [j] | $0.33 \pm 0.06$ | $13 \pm 4$ | $0.17 \pm 0.03$ | $14 \pm 5$ | II | $k_1$ small | – |
| Yeast 3-*O*-Me-glucose | $1.52 \pm 0.12$ | $1.89 \pm 0.14$ | $0.45 \pm 0.06$ | $2.95 \pm 0.19$ | V | $k_1 = k_2$, both small | – |

[a] Side 1 is the cytoplasmic side. Resistances are ± S.E.
[b] Whether or not confirmed in analysis cited.
[c] $R$ in min/mM. Data of Cabantchik and Ginsburg [6], analysed by Lieb [2].
[d] $R$ in (h/mM) × 100. Data of Jarvis et al. [7], analysed by Stein [3].
[e] $R$ in S/mM. Various sources, from table 4.9 of Stein [3], there analysed.
[f] $R$ in min/mM. Data of Hoare [9,10], analyzed by Lieb [2].
[g] $R$ in min/mM. Data of Rosenberg [11], there analysed.
[h] $R$ in min/mM. Data of Rees and Gliemann [8]. Present analysis original.
[i] $R$ in (g protein)·min·$\mu$mol$^{-1}$. Data of White and Christensen [12], analysed by Stein [3].
[j] $R$ in (h·ml·$\mu$mol$^{-1}$) × 100. Data of Komor et al. [13], analysed by Stein [3].
[k] $R$ in (liter cell water)·min·mmol$^{-1}$. Data of Miller and Harun [14], analysed by Stein [3].

cies are found, it seems safe to use the interpretations provided, for the detailed characterisation of transport systems whose behaviour is, by appropriate tests [2], not inconsistent with that of the simple carrier. (Even if it is found that the available data fit the predictions based on the carrier, this does not mean that all the systems so analysed are indeed carriers. No kinetic test can prove that a particular model is applicable – one can only reject models by kinetics [2].)

The presently available data seem to fit into two main patterns. In all cases it is one (or both) of the transformation steps of the unloaded carrier that is rate-limiting for overall transport. One pattern of resistances, the pattern $A=B \gg C, D$ is, marginally, the more prevalent and it appears that it is the rate of one conformation change of the unloaded carrier (generally that in the cytoplasm to exterior direction) that, often, is exceptionally slow. In the other commonly-found pattern, $A=2B=2CD \gg D$, both lumped resistances describing the interconversion of the two forms of the unloaded carrier are large and nearly equally so. Slow rates of some conformation change among the steps that interconvert the loaded carrier seem to be a common feature of membrane transport systems, the exchange-only systems (the antiporters) being extreme cases of this general tendency.

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