

## Use of Current-Voltage Diagrams in Locating Peak Energy Barriers in Cell Membranes

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*Summary.* The current-voltage relations obtained by integrating the Nernst-Planck equations for a variety of energy profiles are obtained. A simple and approximate method for comparing these relations is described. The method is based on using a linearized transform of current-voltage relations for an Eyring single barrier model. A parameter,  $\gamma$ , related to the location of the single barrier in the Eyring model, and to the shape of the barrier in other models, is readily obtained from the slopes of the linearized relations. It is then a simple matter to determine whether a given current-voltage relation allows discrimination between any particular energy profiles. The results show that the equivalent Eyring model does not always place the peak energy barrier in the same position as other models and that quite large errors in the assignment of position may be made if such a model is used. The results are also used to test the ability of some experimental current-voltage diagrams to discriminate between various energy profiles.

Recently, considerable interest has been shown in attempting to locate the positions of the rate-limiting energy barriers to ion movement across cell membranes and artificial membranes. Woodhull (1973) and Hille (1974; 1975) have used current-voltage diagrams and the voltage dependence of proton blockage to locate the major energy barrier to sodium movement through the TTX-sensitive sodium channels in nerve. They conclude that this barrier is located about 25% of the distance across the membrane, being closer to the outside than to the inside surface.

In the case of artificial membranes, various authors (e.g., Haydon & Hladky, 1972; Hall, Mead & Szabo, 1973; Hladky, 1974; Andersen & Fuchs, 1975) have investigated the dependence of the current-voltage relation on the shape of the energy profile across the membrane.

One of the major difficulties in this kind of work lies in the fact that the information contained in a single current-voltage diagram is limited and is usually less than is required to unambiguously describe the shape and magnitude of the energy profile. Even very simple models (such as the single barrier model described in Noble, 1972) may generate a wide

variety of current-voltage diagrams so that it is likely that a variety of significantly different energy profiles may give rise to current-voltage diagrams whose shapes are virtually indistinguishable experimentally. In this paper we shall investigate the extent to which this problem may give rise to difficulties in attempts to locate the position of peak energy barriers. We shall do this by comparing the current-voltage diagrams produced by integrating the Nernst-Planck equations for various energy profiles with those produced by a simple single barrier model.

We shall show that quite large errors are possible in determining the location of peak barriers if no additional information on the energy profile is available and if the “wrong” profile is chosen for analysis.

## Theory

### *Equations for Single Barrier Model*

The models we shall consider here share three features in common. First, the voltage profile across the membrane is assumed to be linear. Secondly, the energy profile in the presence of an electrical field is assumed to be formed at each point by the algebraic sum of the energy at zero electric field and the energy of the field itself. This assumption is equivalent to saying that the electrical field itself does not change the non-electrical energy barriers. Thus, no gating reactions are involved; we are concerned solely with the ion transfer reaction in the absence of electrical gating. Finally, we shall assume that the independence principle holds in all cases. The barriers to ion movement are not dependent on the ion movement itself.

The reasons for making these assumptions are not that we consider them to be realistic—in the case of real excitable membranes they clearly are not realistic in most cases—but rather because we wish to investigate whether there is a considerable degree of ambiguity in interpreting current-voltage diagrams, even when these simplifying assumptions are made. The ambiguity will only increase further if more complex models are investigated, except when these models result in current-voltage diagrams that actually lie *outside* the range of diagrams given by simpler models.

This range may readily be established by considering the simplest model: the single rate-limiting Eyring energy barrier. This barrier is assumed to lie at a distance from the surface of the membrane given by

a parameter  $\gamma$  that may vary between 0 (rate-limiting barrier on surface on side 1) and 1 (rate-limiting barrier on a surface on side 2).  $\gamma=0.5$  thus corresponds to a barrier placed exactly in the centre of the membrane. The equation for the steady-state current-voltage diagram in this case is readily obtained from Eyring rate theory. When equal ion concentrations occur on each side of the membrane, i.e.,  $C_1 = C_2$ , and only monovalent species are considered, we obtain (*cf.* Noble, 1972):

$$I = AFC e^{-G/RT} (e^{\gamma EF/RT} - e^{-(1-\gamma)EF/RT}) \quad (1)$$

where  $I$  is the current,  $A$  is a constant,  $F$  is the Faraday,  $C$  is the concentration of ions,  $G$  is the magnitude of the peak energy barrier in the absence of an electrical field,  $R$  is the gas constant,  $T$  is absolute temperature,  $E$  is the transmembrane potential, and  $\gamma$  is the "position" parameter discussed above.

Fig. 1 (*top*) shows the current-voltage diagrams given by Eq. (1) for  $\gamma=0.05$ , 0.5 and 0.95. When  $\gamma=0.05$ , the relation is concave downwards (towards the  $E$  axis) and becomes flat at large potentials. When  $\gamma=0.95$ , the relation is concave upwards and the current simply increases indefinitely with potential. All the current-voltage diagrams investigated in the models described in this paper must lie between those for  $\gamma=0$  and  $\gamma=1$  although their shapes might or might not be well described by Eq. (1). In order to compare the various models we shall determine which value of  $\gamma$  in the single energy barrier model gives the closest fit to the current-voltage diagrams. This may be done by using a plot that linearizes all current-voltage diagrams given by Eq. (1) so that the slope of the line will give a measure of  $\gamma$  and the degree of linearity will give a measure of the goodness of fit.

The linearization may be achieved as follows: Eq. (1) may be rewritten.

$$I = AFC e^{-G/RT} e^{\gamma EF/RT} (1 - e^{-EF/RT}). \quad (2)$$

If we compare currents at two potentials,  $E$  and a reference potential,  $E_0$ , we obtain

$$\frac{I_0}{I} = e^{\gamma(E_0 - E)F/RT} \cdot \left( \frac{1 - e^{E_0 F/RT}}{1 - e^{EF/RT}} \right) \quad (3)$$

and we obtain

$$(E_0 - E) = \frac{2 \cdot 3}{\gamma F/RT} \log_{10} \left[ \frac{I_0}{I} \frac{(1 - e^{-EF/RT})}{(1 - e^{-E_0 F/RT})} \right]. \quad (4)$$

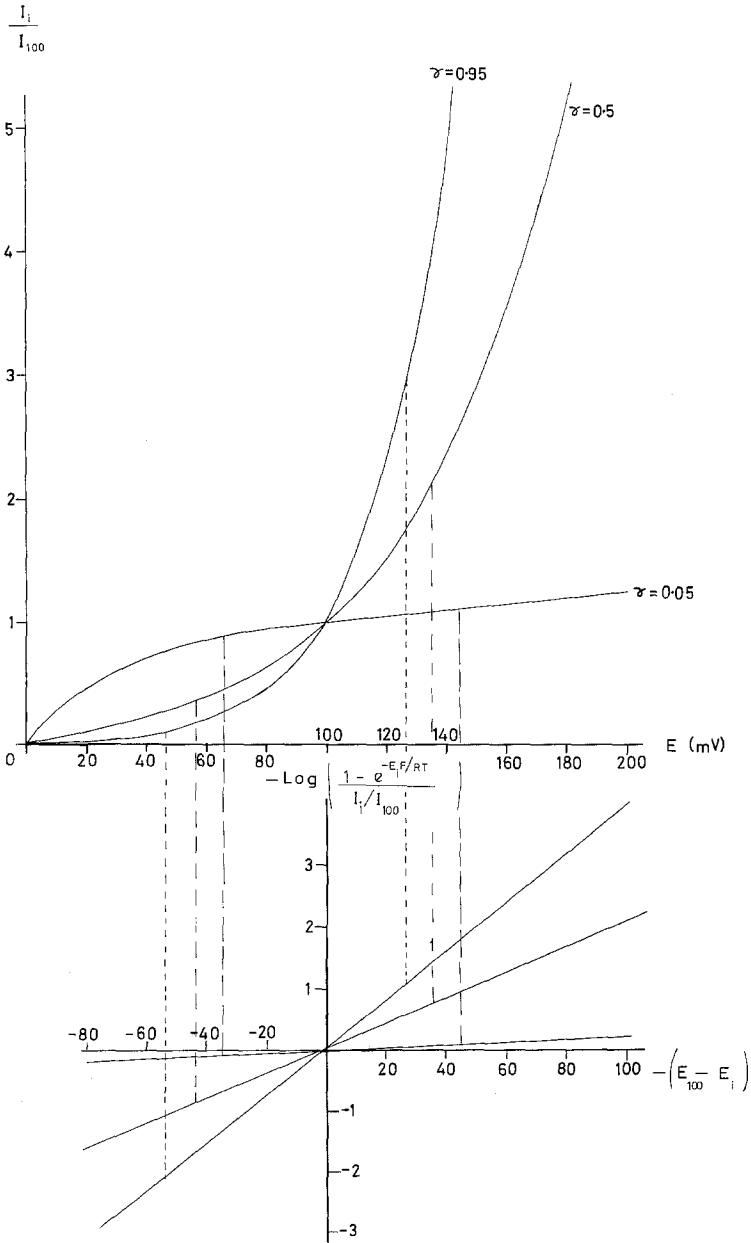


Fig. 1. *Top*: Current voltage relations derived from the single rate limiting Eyring barrier positioned close to either surface ( $\gamma=0.05, 0.95$ ) or the middle ( $\gamma=0.5$ ) of the membrane. *Bottom*: Linearized plots of the top curves

If we normalize  $I$  relative to  $I_0$  then

$$(E_0 - E) = \frac{2.3}{\gamma F/RT} \log_{10} \left( \frac{1 - e^{-EF/RT}}{I/I_0} \right) + \text{constant}. \quad (5)$$

The constant is  $\frac{-2 \cdot 3}{\gamma F/RT} \log_{10} (1 - e^{E_0 F/RT})$ . We shall usually take  $E_0$  to be 100 mV. This choice is arbitrary but immaterial. Any other choice of  $E_0$  would do, although for accuracy in normalizing  $I$  it is better to choose a relatively large value of  $E_0$ .

Thus, the single barrier model predicts that a plot of  $\log_{10} \left( \frac{1 - e^{-EF/RT}}{I/I_0} \right)$  against  $(E_0 - E)$  should yield a straight line whose slope is given by  $\gamma F/2 \cdot 3 RT$ . Fig. 1 (*Bottom*) shows the results of Fig. 1 (*top*) replotted in this manner. These kinds of plots are equivalent to the use of Tafel's equation in electrochemistry (see Kortüm, 1965, p. 467). Our  $\gamma$  parameter is exactly analogous to the transition factor,  $\alpha$ , in Tafel's equation.

### Equations for Other Energy Profiles

(a) *Trapezoid barrier.* One of the energy profiles of interest in work on ion transport by hydrophobic carriers, such as valinomycin and monactin, is that attributable to the electrostatic energy required to carry a charged particle through a medium of low dielectric constant. This energy may be of the order of 30–50 kcal (see Neumcke & Läuger, 1969; Haydon & Hladky, 1972; Brown, 1974) and activation enthalpies of this order of magnitude have been measured experimentally (Ginsburg & Noble, 1974). In this case, the energy profile expected is one in which the peak energy is reached quite rapidly on entering the hydrophobic phase and then forms a plateau before falling steeply on the other side of the hydrophobic phase (*cf.* Brown, 1974). A simple approximation to this case is given by considering a trapezoidal energy barrier (Haydon & Hladky, 1972; Hall, Mead & Szabo, 1973; Hladky, 1974). In this case we shall use the parameter  $\gamma_T$  to represent the distance at which the edge of the plateau is situated (*see* Fig. 2*a*). This model has the advantage that it may readily be adapted to cases in which the peak electrostatic energy is reached more slowly, as would happen, for example, if the regions of membrane near the surface are more polar than those at the centre. A larger value of  $\gamma_T$  would then be obtained.

One might expect that the best-fitting discrete barrier model in this case would be one in which single energy barriers are placed at the edges of the trapezoid plateau<sup>1</sup>. This is the approximation made by Hall *et al.*

1 It should be noted that the use of two Eyring energy barriers to represent the trapezoid strictly invalidates the use of a single barrier equation. The latter should be asymmetric since if, for positive potentials,  $\gamma = x$  then for negative potentials  $\gamma = x + \gamma_T$ . However, for a two barrier model the rate-limiting barrier will depend on the direction of the field and the same value of  $\gamma$  applies. The degree to which this approximation is valid is tested below (*see* Fig. 3*c*).

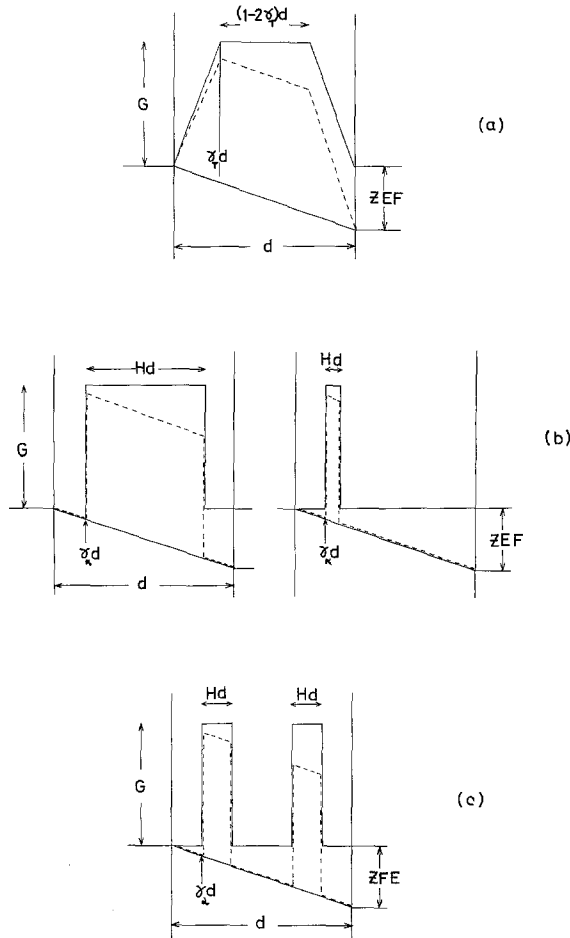


Fig. 2. (a) Trapezoid Barrier; (b) Single Rectangle Barrier; and (c) Double Rectangle Barrier. The membrane thickness is denoted by  $d$ ;  $\gamma$  is a position parameter and  $H$  is a rectangle width parameter

(1973) in considering this particular model (see their paper, p. 81, footnote). This approximation may be tested by comparing the  $\gamma_T$  values for the trapezoid model with those obtained by the best fitting Eyring model.

We have performed this comparison, first obtaining current-voltage relations for the trapezoid profile by integrating the Nernst-Planck equation in the steady state (Haydon & Hladky, 1972, Eq. 27):

$$I = -FD \frac{C_2 e^{G_2/RT} - C_1 e^{G_1/RT}}{\int_0^d e^{G/RT} dx} \tag{6}$$

where  $F$  is the Faraday,  $D$  is the diffusion constant of the charge carrier and  $G$  is the sum of the potential energy  $G_0$  in the absence of a field and the energy of the field:

$$G(x) = G_0(x) + zFE x/d, \quad (7)$$

$d$  is the thickness of the membrane, and  $x$  is the membrane thickness coordinate.  $G_1$  and  $G_2$  are the total energies at the membrane surface 1 and 2 ( $x=0$  and  $x=d$  respectively).

For the trapezoid case, Eq. (6) may be rewritten:

$$I = -FD \frac{C_2 e^{G_2/RT} - C_1 e^{G_1/RT}}{\int_{x=0}^{x=\gamma_T d} e^{G/RT} dx + \int_{x=\gamma_T d}^{x=(1-\gamma_T)d} e^{G/RT} dx + \int_{x=(1-\gamma_T)d}^{x=d} e^{G/RT} dx}, \quad (8)$$

where  $G_1=0$ ,  $G_2 = -zEF = -EF$  (for  $z=1$ ) and

$$G(x) = \begin{cases} \left( \frac{G_0}{\gamma_T} - EF \right) \frac{x}{d} & 0 \leq x \leq \gamma_T d \\ G_0 - \frac{EFx}{d} & \gamma_T d \leq x \leq (1-\gamma_T)d \\ \left( \frac{G_0}{\gamma_T} - \left( \frac{G_0}{\gamma_T} + EF \right) \right) \frac{x}{d} & (1-\gamma_T)d \leq x \leq d. \end{cases} \quad (9)$$

The integrals in Eq. (8) have analytical solutions. We used a computer program to calculate the current voltage relations from Eq. (8) and (9) for trapezoids of different plateau thicknesses (by varying  $\gamma_T$ ) and heights (by varying  $G_0$ ) over the voltage range 0–200 mV.

(b) *Rectangular barrier.* A rectangular barrier extending over a large portion of the membrane (Fig. 2b) may be used as another approximation to the electrostatic barrier encountered by a charged particle crossing a low dielectric membrane.

Narrow rectangular barriers (Fig. 2b) have a second use; the current voltage relations of a very narrow rectangular barrier should be similar to those generated by the simple Eyring rate limiting barrier. In this case, it is of interest to determine how narrow a sharp rectangle must be in order to obtain from the integration of the Nernst Planck equation a current voltage relation virtually identical to the one obtained from the commonly employed Eyring barrier approximation.

In this case, the Nernst-Planck relation is

$$I = -FD \frac{C_2 e^{G_2/RT} - C_1 e^{G_1/RT}}{\int_{x=0}^{x=\gamma_R d} e^{G/RT} dx + \int_{x=\gamma_R d}^{x=(\gamma_R+H)d} e^{G/RT} dx + \int_{x=(\gamma_R+H)d}^{x=d} e^{G/RT} dx} \quad (10)$$

where  $\gamma_R$  is a parameter determining the position of the barrier in the membrane and  $H$  is the rectangle's thickness (see Fig. 2*b*), and

$$G(x) = \begin{cases} G_0 - EFx/d & \gamma_R d \leq x \leq (\gamma_R + H)d \\ -EFx/d & 0 \leq x < \gamma_R d; (x+H)d < x \leq d \end{cases} \quad (11)$$

The  $I(V)$  relations were computed for various rectangular positions, heights and thicknesses.

(*c*) *Double rectangular barrier.* If more than one rate limiting barrier is present in the membrane it is possible to calculate the current voltage relations by using standard rate theory. Eyring and Eyring (1965) have given a general solution to the problem. This approach has a serious limitation in that the concentrations of the permeating species are assumed to be equilibrated between each pair of barriers.

The Nernst-Planck equations eliminate this assumption. We chose the simplest case, a double rectangular barrier and calculated the current voltage relations generated for various heights and thicknesses of symmetric double rectangular barriers (Fig. 2*c*).

The current is given by

$$I = -FD \frac{C_2 e^{G_2/RT}}{\int_{x=0}^{x=\gamma_2 d} e^{G/RT} dx + \int_{x=\gamma_2 d}^{x=(\gamma_2+H)d} e^{G/RT} dx + \int_{x=(\gamma_2+H)d}^{x=(1-\gamma_2-H)d} e^{G/RT} dx} - \frac{C_1 e^{G_1/RT}}{\int_{x=(1-\gamma_2-H)d}^{x=(1-\gamma_2)d} e^{G/RT} dx + \int_{x=(1-\gamma_2)d}^{x=d} e^{G/RT} dx} \quad (12)$$

$G$  is given by

$$G(x) = \begin{cases} G_0 - \frac{EFx}{d} & \gamma_2 d \leq x \leq (\gamma_2 + H)d \\ & (1 - \gamma_2 - H)d \leq x \leq (1 - \gamma_2)d \\ -\frac{EFx}{d} & 0 \leq x < \gamma_2 d \\ & (\gamma_2 + H)d < x < (1 - \gamma_2 - H)d \\ & (1 - \gamma_2)d < x \leq d. \end{cases} \quad (13)$$



It has been suggested (Hall *et al.*, 1973) that a double-barrier may serve as an approximation to the trapezoid barrier. The double rectangular barrier will be used to test this approximation.

## Results and Discussion

### *Calculated Current Voltage Relations*

All the barriers discussed above produce nonlinear current voltage diagrams with various curvatures, depending on the particular choice of  $G_0$ ,  $\gamma$  and  $H$ .

In order to determine which barriers can account for an experimental  $I(V)$  curve, we have compared the calculated  $I(V)$  relations with the linearized single Eyring barrier relation (Eq. (5)).

The computed currents at various voltages for the different barriers were used to plot  $(E_0 - E)$  vs.  $\log_{10} \left( \frac{1 - e^{-EF/RT}}{I/I_0} \right)$ . The plots were usually nonlinear. The best fitting straight line through the points of the plot gives the best  $\gamma$  value for the single Eyring barrier that can be correlated with the original barrier.

Thus, for example, if the computed  $I(V)$  values of a trapezoid barrier 5 kcal high and of a plateau thickness  $0.6d$  (which means  $\gamma_T = 0.2$ ) are used to plot  $(E_0 - E)$  vs.  $\log_{10} \left( \frac{1 - e^{-EF/RT}}{I/I_0} \right)$ , and a least squares method is employed to fit a straight line through the computed points we find that this particular straight line describes precisely an Eyring barrier positioned such that  $\gamma_{\text{Eyring}} = 0.35$ , which is nearly twice  $\gamma_T$ .

In Fig. 3a the best fitting  $\gamma$  values for the single Eyring barrier are plotted against trapezoidal barrier  $\gamma_T$  values. In Figs. 3b and c, similar  $\gamma$  values are plotted against the values of rectangular ( $\gamma_R$ ) and double ( $\gamma_2$ ) rectangular barriers.

The actual "linearization plots" (based on Eq. 5) are given in Figs. 4, 5, and 6.

As would be expected, only the single rectangle barrier linearized plots (Fig. 6) lie on the best fitting straight lines. Both the trapezoidal and the double rectangular linearized plots deviate from the best fitting straight lines. As seen in Figs. 4 and 5, both deviations are such that the plots are convex in relation to the voltage axis. When  $\gamma$  reaches the value

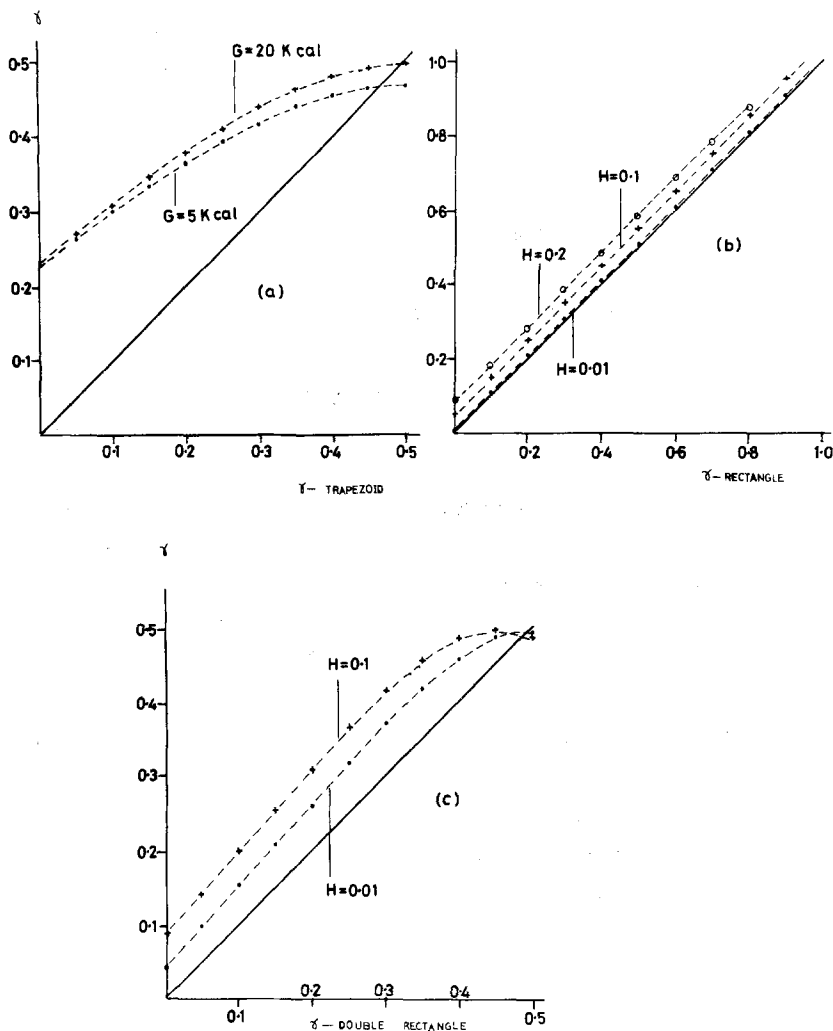


Fig. 3. Relationship between the position parameter  $\gamma$  of a given barrier and its best fitting Eyring-model position parameter  $\gamma_{ffr}$ . (a) Trapezoid barrier; (b) Rectangle barrier; and (c) Double Rectangle barrier

0.30, however, the plots virtually coincide with the best fitting straight lines.

One may therefore conclude that if the actual barrier across the membrane is a trapezoid with a plateau thickness larger than 40% of the membrane thickness or consists of two distinct rectangles separated from each other by a distance larger than 40% of the membrane thickness, the linearized current voltage relation will readily reveal the situation (although a distinction between these two different barriers, i.e., trapezoid or double

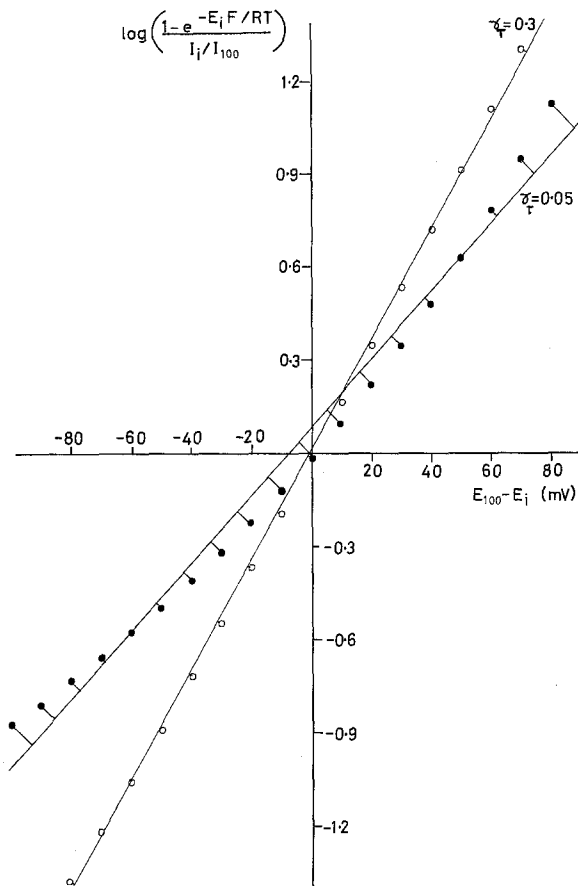


Fig. 4. Linearized current voltage curves for a wide ( $\gamma_T=0.05$ ) and a narrow ( $\gamma_T=0.30$ ) trapezoid barrier, 10 kcal in height. The points are derived from the integrated solutions of the Nernst-Planck equation. The lines are the best fitting straight lines, least squares fitted by computer. (The plots for 5 and 20 kcal trapezoid heights are similar but not identical)

rectangle, may not be possible). The linearized plots will be convex towards the voltage axis in both cases and will not coincide with best-fitting straight lines. (On an ordinary current-voltage diagram such a situation will be expressed by a less “steep” current voltage relation relative to a single rectangle or a single Eyring barrier. In other words, the current increase with voltage will be more gradual in the case of a trapezoid or double rectangle barrier. The linearized plots express this difference clearly, since the single rectangle or the Eyring barriers yield perfect straight lines while the trapezoid and double rectangle are curved).

If, however, the trapezoid plateau is small, or if the two rectangular barriers are close to each other (i.e., in both cases  $\gamma > 0.30$ ) the linearized

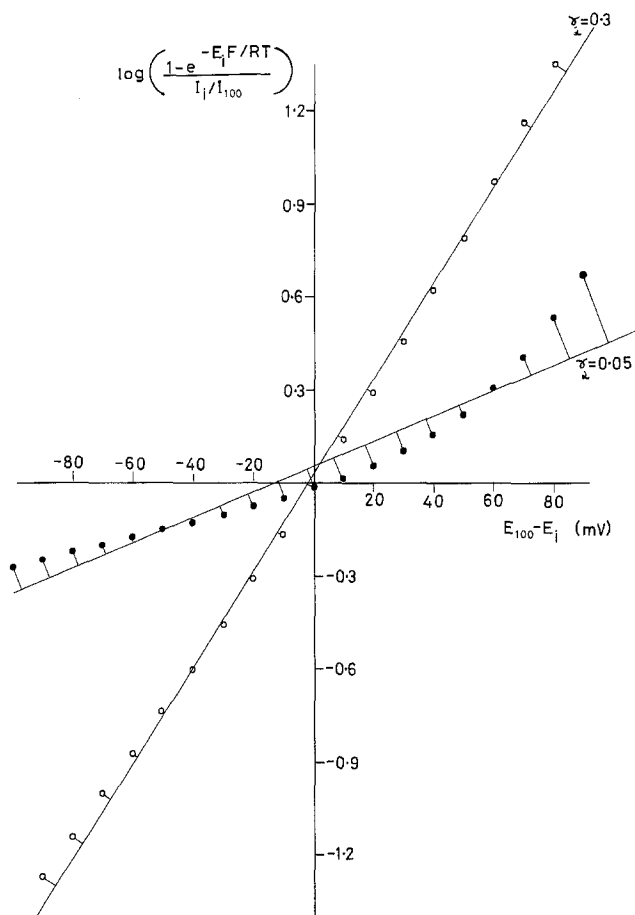


Fig. 5. Linearized current voltage curves for double rectangle barriers  $\geq 5$  kcal high, and 1% thick (i.e.  $H=0.01$ ). Points derive from Nernst-Planck solutions, lines are least-squares-best-fitting. (For  $H=0.10$  the plots are similar, but not identical)

plots will form nearly straight lines. It will be impossible to know on the basis of the current voltage relations whether these straight lines correspond to a specific barrier. For example, reading off Fig. 3, a linearized current voltage fitting a straight line with a slope corresponding to  $\gamma=0.42$  is compatible with any one of the following situations:

- (1) A single Eyring barrier (of any height) with  $\gamma=0.42$ .
- (2) A single rectangle barrier (of any height  $\geq 3$  kcal) with  $\gamma_R=0.34$  (for rectangle thickness  $H=0.20$ ), with  $\gamma_R=0.39$  (for  $H=0.10$ ) or with  $\gamma_R=0.42$  (for  $H=0.01$ ).
- (3) A 5–10 kcal high trapezoid barrier with  $\gamma_T=0.30$ .
- (4) A double rectangle barrier (of any height  $\geq 5$  kcal) and  $H=0.01$  with  $\gamma_2=0.35$ .

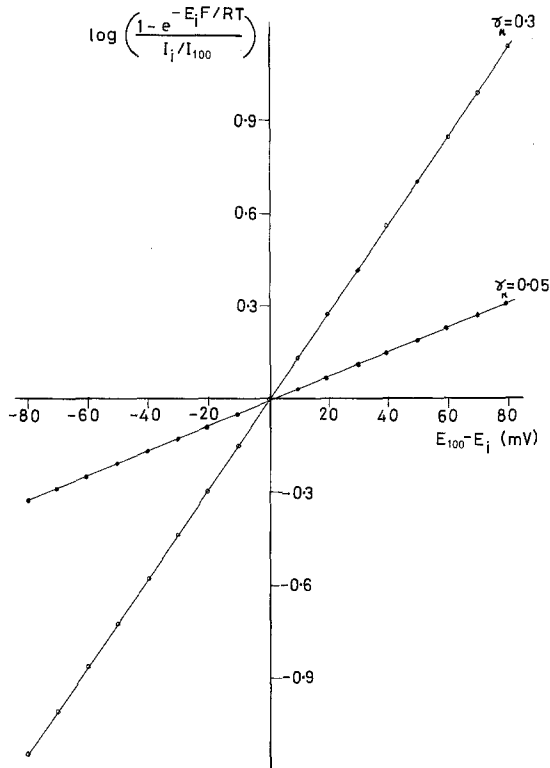


Fig. 6. Linearized current voltage curves for single rectangle barriers  $\geq 3$  kcal high with  $H=0.01$ . The least-squares best fitting straight lines coincide with the points which were calculated using the Nernst-Planck equation. (For  $H>0.01$  the plots are similar, up to  $H=0.10$ . Beyond that they deviate significantly)

### *Specific Examples of Current Voltage Relations*

We have used the current voltage relations of Hall *et al.* (1973) who studied the nonactin mediated transport of potassium ions across lipid bilayers to calculate the best fitting straight line in the linearization plot. The results were taken from the points in Fig. 9 of Hall *et al.* which describes the  $I(V)$  curve in the presence of 3 symmetric KCl solutions.

Fig. 7 shows the "linearized" plots. The points deviate from a straight line and on this basis a single Eyring or rectangle barrier is excluded, in agreement with Hall *et al.* These authors concluded that their results could be fitted with a trapezoid barrier of  $\gamma_T=0.28$ .

In contrast, we find that their experimental current voltage relations fit lower trapezoid  $\gamma_T$  values. Our fitting process is based on comparing

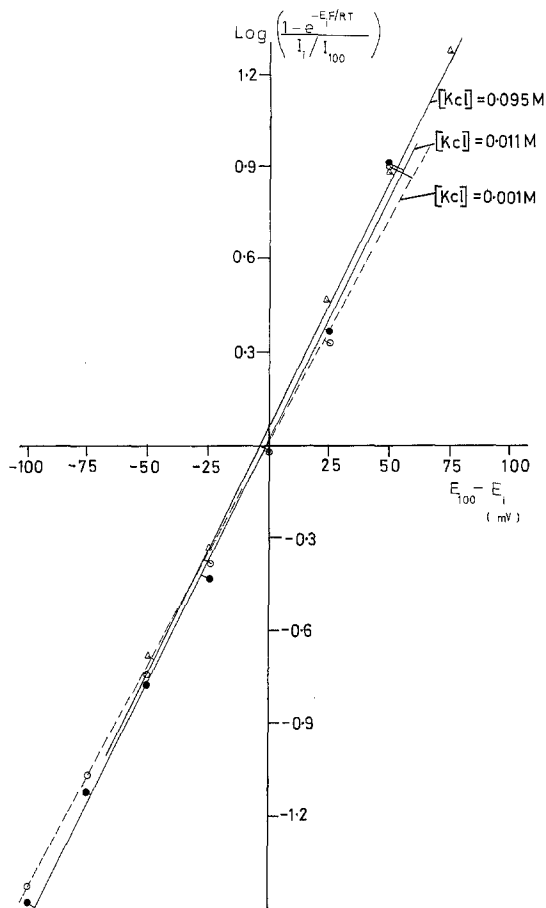


Fig. 7. The results of Hall, Mead and Szabo (1973) (points) replotted, with the least-squares best fitting linearized current voltage lines (straight lines). The results refer to nonactin mediated  $K$  transport across phosphatidyl-ethanolamine bilayers

current voltage relations generated by the integration of the Nernst-Planck equation for trapezoid barriers with the experimental  $I(V)$  relations using the linearization process. We find that the nonactin results with  $[KCl]=0.095$  and  $[KCl]=0.011$  M fit trapezoids with  $\gamma_T=0.225$ , 0.210 and 0.200 for barrier heights 5, 10 and 20 kcal, respectively.

The difference between these estimates and that of Hall *et al.* is not, however, significant since it is attributable to different ways in which the edge is defined. A sharp edged trapezoid that closely fits Hall *et al.*'s function (their equation 28) requires  $\gamma=0.22$ . But there is a very large difference between these values and the Eyring approximation, which

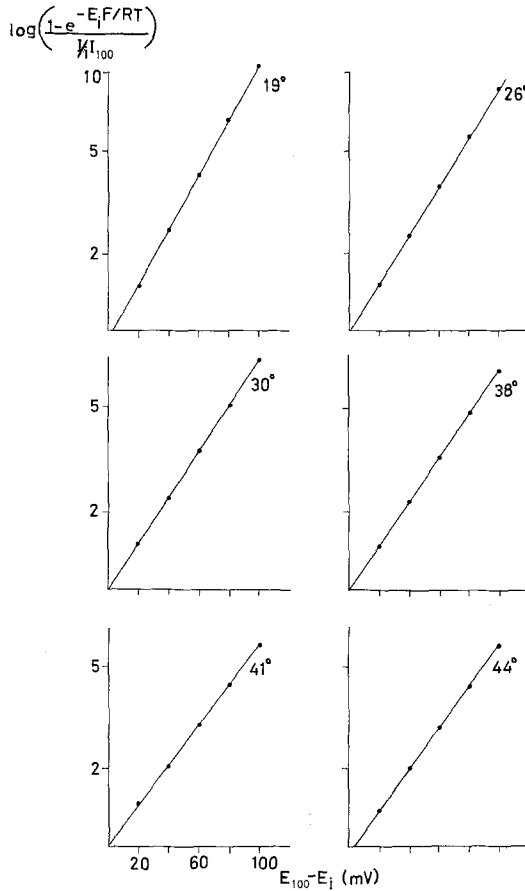


Fig. 8. Linearized current voltage relations (*points*) for 18-crown-6 mediated Na transport across phosphatidyl-serine bilayers, at different temperatures. The points coincide with the least-squares fitted straight lines

from Fig. 3(a) would require  $\gamma = 0.38$ . The Eyring equivalent, therefore, grossly underestimates the plateau thickness.

We have studied the temperature dependence of the current-voltage relation of 18-crown-6 mediated sodium transport in phosphatidyl-serine bilayers. The linearized current-voltage relations of this system are given in Fig. 8. At six different temperatures the  $\gamma$  values are 0.61 (19 °C), 0.56 (26 °C), 0.535 (30 °C), 0.52 (38 °C), 0.49 (41 °C) and 0.51 (44 °C). It appears, therefore, that in this case a steep energy barrier located in the middle of the membrane ( $\gamma = 0.5$ ) is responsible for the current voltage relations at all temperatures.

The origin of trapezoid (or approximately trapezoid) barriers in membranes is the "image barrier" which is the potential energy of a charged

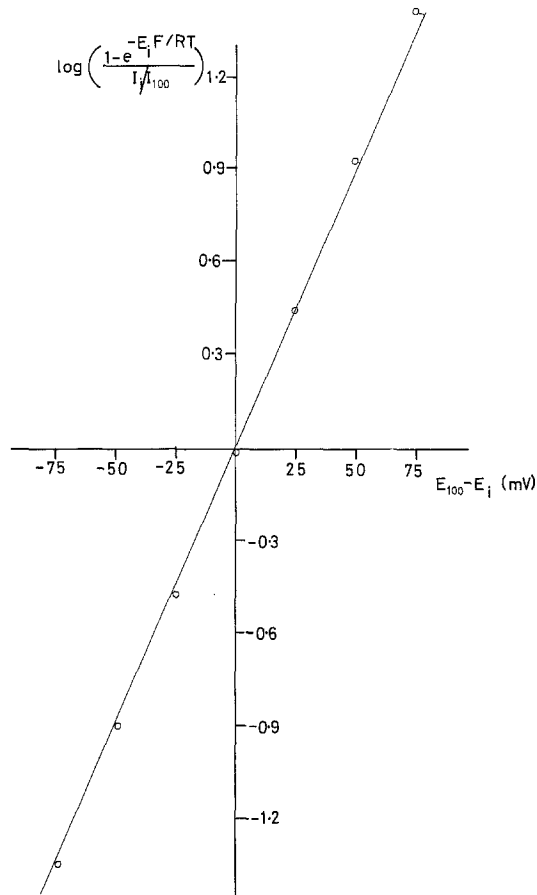


Fig. 9. The image charge barrier calculations of Haydon and Hladky (1972) replotted (*points*) and the best-fitting linearized current-voltage line

species in a thin, low, dielectric medium (membrane) separating two semi-infinite high dielectric media (the aqueous solutions). Haydon and Hladky (1972) calculated the shape of this barrier and the current-voltage relation expected from the energy profile across the membrane. They found that the calculated conductance-voltage relation (Table B-1 in their paper) fits their experimental data for sodium-nonactin bilayer conductances.

We have used their current voltage calculations (Eq. B-17 and Table B-1) in the linearization plot. We find (*see* Fig. 9) that the image barrier linearized current-voltage values fall very nearly on a best fitting straight line with a slope corresponding to  $\gamma = 0.44$ .

Thus, the ambiguity of current-voltage interpretation is demonstrated once more. On the basis of current-voltage relations the calculated values



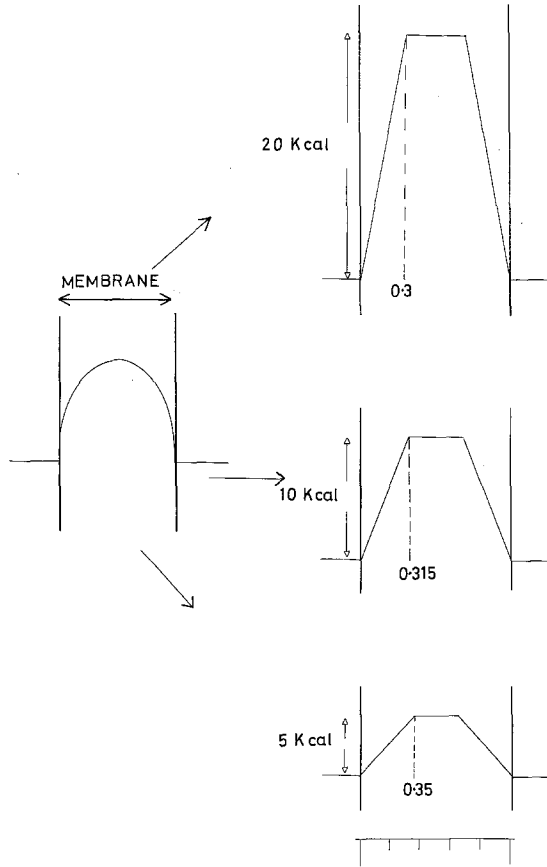


Fig. 10. Correlation between image-charge and trapezoid barriers

(or indeed the experimental Na-nonactin data) are compatible with two very different energy profiles: an image charge barrier and a single Eyring barrier situated at a distance 0.44 from the membrane surface. While this ambiguity holds for the experimental sodium-nonactin results, it does not, of course, hold for the theoretical image barrier values which were specifically calculated on the basis of a given energy profile across the membrane.

The image barrier generates current-voltage relations that may be compared with those generated by theoretical trapezoid barriers. We find that the trapezoids which approximate the image barrier best are (Fig. 10): a trapezoid 5 kcal high with a plateau thickness 0.30; a trapezoid 10 kcal high with thickness 0.37 or trapezoids 20–50 kcal high with plateau thickness 0.40.

## Conclusions

The general conclusion of this paper is that the ability of a current-voltage diagram to distinguish between various energy profiles is limited. The degree of limitation depends on the shape of the relation and estimates of the position of peak energy barriers may then depend on which profile is chosen for analysis. Finally, our results show that it is not valid to use Eyring "equivalents" for some profiles since the intuitive notion that the equivalent Eyring barriers should be placed at the edges of plateau regions is usually incorrect.

These conclusions apply to energy profiles that do not allow steady state conditions to be approached at any point in the membrane. The concentration of ions is then always a function of the current flow. By contrast, models involving deep energy wells (corresponding to strong binding sites) may allow the concentration to approach steady state values before the ions surmount the peak energy barrier. This is the kind of model used by Hille (1974; 1975) for the sodium channel of excitable membranes. The concentration at the binding site is then a much simpler function of its location,  $\gamma$ , and of the potential,  $E$ . An unambiguous assignment of the value of  $\gamma$  then becomes possible. This lack of ambiguity depends, of course, on prior knowledge of the shape of the energy profile. Our general conclusion that current-voltage diagrams by themselves may be inadequate is still applicable.

These conclusions reinforce the need to obtain further empirical information over and above that provided by current-voltage diagrams alone. Anderson and Fuchs (1975) have recently reported the use of tetraphenylborate as a tool for investigating the energy profile in the membrane. The ion movement in this case differs from all the cases considered in this paper inasmuch as the ion movement is entirely confined to the membrane interior and produces current transients that resemble those of the gating currents in excitable membranes. In this case, the measurement of current transients, together with an estimate of the fraction of the electric field influencing transport, provide sufficient additional information to allow discrimination, between, e.g., image force and trapezoidal barriers, to be made.

## References

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