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A Transition State Theory Approach to the Kinetics of Conductance Changes in Excitable Membranes

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Summary. The kinetics of ionic current mechanisms in excitable membranes are analyzed. It is assumed that there are voltage-dependent reactions occurring in the membrane which are independent of the flow of ionic current. The experimental evidence for this assumption is reviewed in the light of more recent results on the kinetics of conductance changes in cardiac membranes. Rate equations are then obtained using transition state theory and assuming that each reaction is rate limited by only one energy barrier. These equations give simple exponential functions for the voltage dependence of the rates. More complex functions may be obtained by assuming that more than one energy barrier is rate limiting. The single-barrier equations are used to estimate the energies of formation of the transition state. In most cases, the entropy of formation is positive but there is no systematic order in the estimated enthalpies. These results are contrasted with those for the ion permeation process itself which normally has a negative entropy of activation. This contrast reinforces the assumption that the reactions controlling membrane permeability are distinct from the ion permeation process itself. The significance of the positive entropy of formation of the transition state in the permeability reactions is discussed, and it is suggested that the membrane structures underlying these reactions may change their degree of hydration during the formation of the transition state.

During the first half of this century, one of the main aims of membrane biophysics was to account for the variations in membrane potential which occur during activity in nerve and muscle cells. This goal has been largely achieved, and in a number of excitable cells the potential changes may now be accounted for quantitatively in terms of transmembrane currents carried by Na, K, and, in some cases, Ca and Cl ions (*see* Hodgkin, 1964; Cole, 1968).

The measurement of these currents was made possible by the invention of the voltage clamp technique (Cole, 1949; Marmont, 1949) which allowed the membrane potential to be an experimentally controlled variable. Use of this technique revealed that, in addition to time, the membrane potential is the crucial independent variable determining the current flow. The potential influences the driving force for the movement of ions, but, more significantly, it also uniquely determines the rate coefficient of current change.

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In other words, the voltage clamp experiments show that the ionic current is dependent on previous membrane potentials, but not on the past history of membrane currents per se. Thus, a characterization of the current response to step changes of potential can be used to predict the current response to any potential waveform, including the currents which flow during the action potential itself.

The discovery of the essential role of membrane potential led Hodgkin and Huxley (1952) to describe the time dependence of membrane currents in squid axon by means of dimensionless variables, each of which obeyed simple first-order kinetics. This elegant formulation was strengthened by ionic substitution experiments which showed that the kinetic variables corresponded to Na-specific and K-specific pathways, respectively. These pathways have often been called Na and K "channels", although it is now known (Chandler & Meves, 1965) that their specificity to cations is not complete. More recently, pharmacological agents which very specifically affect the different channels have provided evidence that the pathways for ionic current are indeed spatially, as well as functionally, distinct (Hille, 1969).

Nevertheless, the molecular nature of the permeation process remains mysterious. It is still not known, for example, whether the ionic pathways correspond to "channels" in the sense of holes or rather to carrier molecules which somehow ferry the ions across the membrane. Nor do we understand the basis of the time and voltage dependence of the ionic current. Does the Hodgkin-Huxley variable correspond to a specialized membrane structure?

A major difficulty in tackling this problem is that, as yet, measurements of the controlling processes cannot be made independently of measurements of the membrane current. The membrane current is a gross summation of the permeation of individual ions, and, furthermore, it is possible that the passage of ions through the membrane might itself influence the structures which are responsible for the voltage sensitivity. Other physical chemical techniques are needed to give more direct information about the nature of such structures.

Despite the inherent ambiguity of current as a probe for membrane structure, the Hodgkin-Huxley kinetics may serve as a goal. Hopefully, other measurements of the state of the membrane (e.g., magnetic resonance spectroscopy) should have components which resemble the current in their time and voltage dependence. Of course, this is only a working hypothesis; until Hodgkin-Huxley variables are actually measured by other techniques, it cannot be regarded as certain that they are anything more than a convenient formalism for obtaining a relatively simple mathematical description of the membrane currents. Moreover, a variety of different mathematical

formulations have been shown to be adequate to describe the membrane currents in squid nerve.

The purpose of this review paper is to discuss the general physico-chemical implications of treating the controlling mechanisms described by the Hodgkin-Huxley kinetics as real membrane structural changes which are independent of the membrane current. We shall also describe a rather different (although not incompatible) physico-chemical approach to the kinetics of these changes to that used by Hodgkin and Huxley. The impetus for this work was provided by our experimental analysis of the voltage clamp currents in cardiac membranes (Noble & Tsien, 1968*a*, 1969*a*, *b*; Hauswirth, Noble & Tsien, 1968, 1969); our second aim is to place these newer results in perspective.

Results

Kinetics of Current Changes in Heart Cells

In the case of squid nerve membrane, the current records can be accounted for by postulating the existence of three voltage-dependent first-order reactions (Hodgkin & Huxley, 1952). The fastest of these, described by the Hodgkin-Huxley variable m, controls the activation of the inward sodium current which depolarizes the membrane. The slower reactions, h and n, control the inactivation of the sodium current and the activation of the outward potassium current, respectively. All these reactions occur within a few milliseconds following step changes in the independent variable, the membrane potential (E_m). Some of the more recent evidence that these reactions are independent has been reviewed by Hille (1969).

As yet, the inward currents in cardiac muscle (sodium and calcium) have not been subjected to a full kinetic analysis, although Weidmann (1955) provided a partial analysis of the sodium inactivation process (h) in Purkinje fibers. The outward currents, however, have been analyzed in some detail, and the results show a number of new features.

First, in the case of Purkinje fibers, there are no less than three (and there may be four) independent current-controlling systems whose kinetics resemble those of the squid potassium current in form (although not, as we shall see, in detail). In frog atrial muscle, the only other cardiac tissue whose outward membrane current kinetics have become available (Brown & Noble, 1969*a*, *b*), there are two such systems which appear to correspond to two of the Purkinje fiber membrane systems. One of the Purkinje fiber components simply appears to be absent. The evidence for the distinctness of these current components has been described in detail elsewhere (Noble & Tsien, 1969; Hauswirth, Noble & Tsien, *in preparation*).

One of the important functional differences between the cardiac currents is that they are activated by different ranges of potential. This is shown in Fig. 1 (top) which plots the steady state degree of activation of each component as a function of the membrane potential. One of the components (labelled s) is activated by potentials in a very negative range (-90 to -60 mV). The other two components (labelled x_1 and x_2) are activated at less negative potentials.

Second, the activation reactions are extremely slow. Thus, the longest time constants are of the order of 2 sec (s), 1 sec (x_1), and 5 sec (x_2) at about 35 °C. These are three orders of magnitude slower than the time constants for the processes underlying nerve activity.

Third, the temperature dependence of the kinetics is very great. In the case of the s reaction, the Q_{10} is about 6, compared to values between 2 and 3 for the nerve reactions.

Finally, in contrast to the "channels" of squid nerve membrane [which behave as linear (ohmic) resistances once activation has occurred], the cardiac "channels" are grossly nonlinear. This is shown in Fig. 1 (bottom) which shows typical current-voltage relations for each of the three components in the fully activated state. Only i_{x_2} is linear. The current components controlled by s and x_1 show marked inward-going rectification; i.e., the s and x_1 channels pass inward current more easily than outward current. This phenomenom is not yet understood (*see* Noble, 1965), although Adrian (1969) has described a possible mechanism.

It is also evident from the current-voltage relations shown in Fig. 1 that the cardiac outward-current channels differ in their ionic selectivity. The s channel has a current-voltage relation which crosses the voltage axis at the K equilibrium potential (about -100 mV), indicating a high K specificity. The other channels have less negative reversal potentials, and are partially permeable to other ions as well as K.

Despite these substantial differences from the nerve membrane currents (some of which are functionally important to the generation of electrical activity in cardiac muscle), it is striking that the kinetics governing s, x_1 and x_2 in heart are formally identical with those of m, n or h in nerve. Thus,

$$\mathrm{d}s/\mathrm{d}t=\alpha_s(1-s)-\beta_s s\,, \tag{1}$$

$$\mathrm{d}x_1/\mathrm{d}t=\alpha_{x_1}(1-x_1)-\beta_{x_1}x_1\,, \tag{2}$$

$$\mathrm{d}x_2/\mathrm{d}t=\alpha_{x_2}(1-x_2)-\beta_{x_2}x_2 \tag{3}$$

where the α's and β's are rate coefficients which are exponential (or sometimes linear-exponential) functions of E_m. In each case, the ionic current

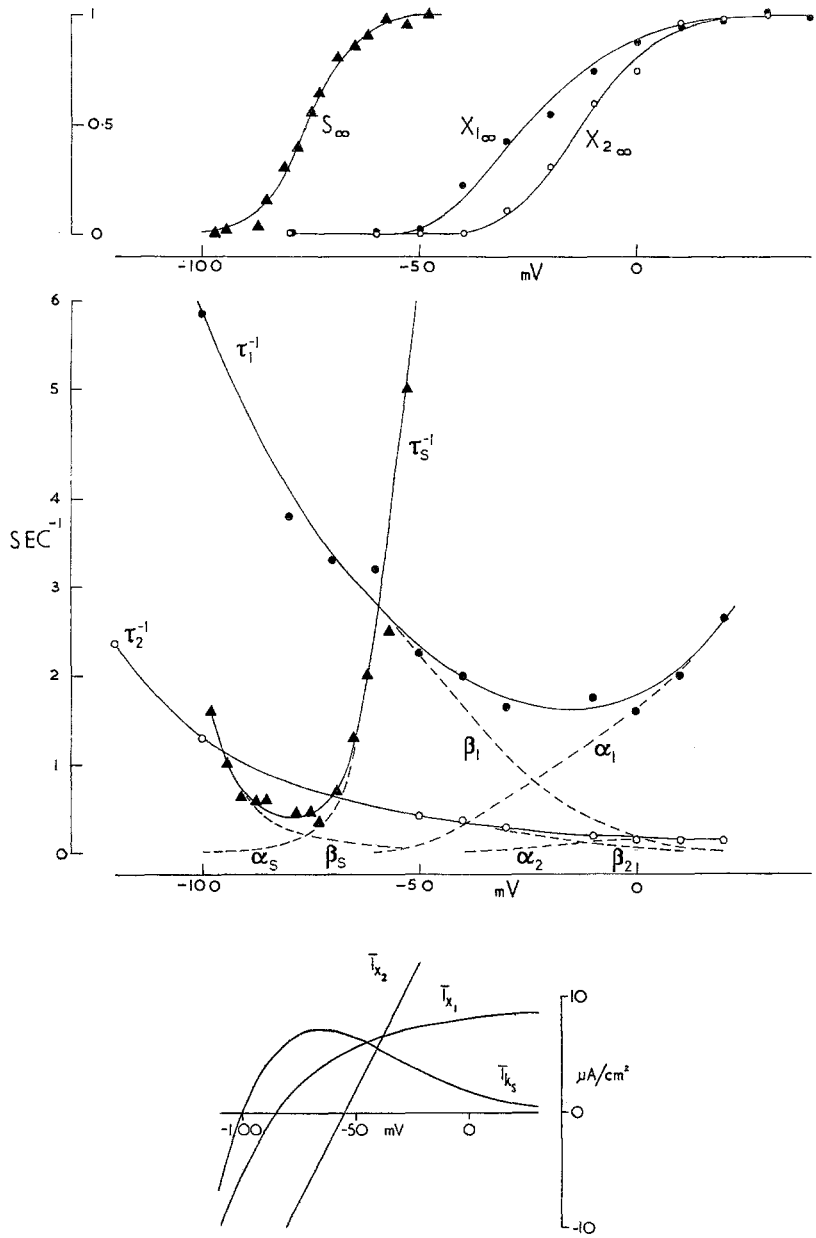


Fig. 1. Kinetics and rectification properties of the slowly activating currents i_{K_s}, i_{x_1} and i_{x_2} in cardiac Purkinje fibers. *Top*: Fractional degree of activation of each component as a function of membrane potential. The s component is activated in the "pacemaker" range of potentials (−90 to −60 mV). The x components are activated in the "plateau" range (−50 to +10 mV). *Middle*: Voltage dependence of rate coefficients in Eqs. (1), (2) and (3). *Bottom*: Voltage dependence of currents when each system is fully activated. s and x_1 channels show inward-going rectification. x_2 displays virtually no rectification. Figure based on Noble and Tsien (1968, 1969)

can be described as the product of the kinetic variable and a term $\bar{i}$ which describes the influence of the electrochemical gradient on current flow.

$$i_{K_s} = \bar{i}_{K_s} \cdot s, \tag{4}$$

$$i_{x_1} = \bar{i}_{x_1} \cdot x_1, \tag{5}$$

$$i_{x_2} = \bar{i}_{x_2} \cdot x_2. \tag{6}$$

The physical significance of this simple description is supported by experiments which demonstrate specific effects on one or other of the factors. For example, as the external potassium concentration is varied (Noble & Tsien, 1968; Peper & Trautwein, *in preperation*), the reversal potential for i_{K_s} follows E_K, but the voltage dependence of s remains unchanged. Conversely, it is possible to influence selectively the voltage dependence of s by altering $[Ca^{++}]_0$ or by adrenaline (Hauswirth, Noble & Tsien, 1968). These results are in line with earlier work in nerve: the voltage and time dependence described by the Hodgkin-Huxley variable is independent of current flow, and, furthermore, the cation selectivity of a channel does not change with the degree of activation (Chandler & Meves, 1965).

The simplest interpretation of these experiments is that the Hodgkin-Huxley variables describe distinct "gating" processes, operating in conjunction at individual channels. It may be sufficient at this point (*see* also Discussion) to distinguish the voltage dependence of the slow current changes from the $\bar{i}$ current-voltage relations. The latter are, by comparison, "instantaneous" (or at least they correspond to processes that are faster than the ca. 50-μsec resolution of the fastest voltage clamp techniques).

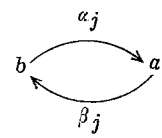
In contrast to nerve, one convenient feature of the cardiac currents is that step changes of potential produce exponential (and not sigmoid) current changes. The currents can therefore be described by Hodgkin-Huxley variables without power relations. This disposes of some of the ambiguity concerning the mathematical formulation: it would be perverse *not* to use first-order differential equations in these cases. The similarity of the kinetics of currents in different excitable membranes supports the view that the Hodgkin-Huxley formulation is physically meaningful; we shall, therefore, continue to use it in this paper.

One further point seems worth making at this stage in the light of the cardiac membrane results. Whereas it is possible, as Mullins (1959, 1968), Agin and Rojas (1963), Goldman (1964), and others have shown, to develop models of the nerve currents which do not represent the three reactions as occuring independently (i.e., "in parallel"), such models would become

extremely cumbersome if applied to cardiac membranes. In addition to the three or four outward current reactions, there are probably four (two activation and two inactivation) reactions controlling the inward currents. It seems much more likely that all the reactions are occuring independently. Moreover, as far as the kinetics of s, x_1, and x_2 are concerned, it is difficult to see how the results may be fitted by any "non-parallel" model.

General Physico-Chemical Implications of Hodgkin-Huxley Kinetics

As Hodgkin and Huxley originally intended it, the kinetic variable gives the probability that a charged structure in the membrane will be in a state which allows a path for ion current flow. Modifying Noble's (1966) terminology, we shall call the conduction state a and the nonconduction state b. Thus, for each reaction we have



where α_j and β_j stand for voltage-dependent rate coefficients. The fraction of structures in the a state is j where j may stand for any of the H-H variables m, h, n, s, x_1, x_2. If it is supposed that a negligible fraction of particles is in transition at a given moment, the fraction in the b state is $l-j$. Equations such as (1), (2) and (3) then readily follow. In this section we shall deal with some of the physico-chemical properties of the reaction.

1. Although each variable, j, varies as a continuous function of potential and time, this does not imply that the conductance of each ionic channel must vary continuously. It is more likely that each channel is quantal in its behavior and that the continuous nature of j is a macroscopic phenomenom. Verveen and Derksen (1968) have observed membrane noise which may be attributable to individual ionic channels for K^+ ions.

2. The rate coefficients do not represent speeds of movement of particles, but rather they represent the average frequency at which structures change from one state to the other. In other words, the time taken for transitions between a and b is very brief in comparison to the average lifetime of the states. In particular, it is important to distinguish the rate coefficients α and β from the rates of movements of ions through the membrane. This is clear from the fact that α and β are obtained by measuring the *rates of change* of ionic currents.

3. Although the formulation is expressed in terms of only two states, it is possible that more than two states may exist chemically. For example, the structure may exhibit a multiplicity of states, b_1, b_2, b_3 which are each nonconducting. The results do not exclude the possibility of a series of transitions; they require only that at least one transition between a conducting and nonconducting state should be rate-limiting. Clearly, this requirement can only be as valid as the evidence for first-order kinetics. In the case of nerve, the first-order kinetics are apparent only when it is assumed that the movement of ions in each channel is controlled by several such processes, operating independently. Thus, the original results were fitted quite closely by assuming that $i_K \alpha n^4$. Later results (Cole & Moore, 1960) required even higher powers – up to 25. The conjunction of such a large number of processes seems physically implausible and raises the question if the basic assumption of a simple first-order process for n might be incorrect. Thus, it might be more plausible to assume that on hyperpolarization (which is the pre-condition for obtaining records which require high exponents) some of the structures may undergo transitions from b to another nonconducting state from which they must return via a rate-limiting transition before becoming available for the b to a transition. This would introduce a further delay in the onset of current, corresponding to the experimental observation.

As we have already noted, the cardiac results are conveniently free of these difficulties. It is a direct observation (the time courses of current change in response to step polarizations are simple exponentials) that $i_K \propto s$ and that the other currents are proportional to x_1 and x_2. In this respect, the cardiac currents are simpler to analyze – a fortunate circumstance since, as noted in the previous section, there are other complications to be overcome in their case.

4. The degree of activation (measured in the steady state condition when E_m is constant and dn/dt or ds/dt, etc., is zero) is usually a very steep function of the membrane potential. For example, in squid nerve, g_{Na} changes e-fold per 4-mV change in transmembrane potential. As Hodgkin and Huxley pointed out, this change requires that a minimum of six unit charges should experience the potential change (an even higher valency is required if only a fraction of the total membrane potential difference is experienced by the charge movement). Thus, since $g_{Na} \propto m^3$, the valency of each "m particle" would be at least two. The movement of these "particles" should therefore produce a transient current whenever their equilibrium distribution is altered by an imposed potential change (*see* Hille, 1969). Such a current has not been detected (Chandler & Meves, 1965). Moreover,

it is unlikely on quantitative grounds that it should be. Thus, recent estimates of the density of sites for sodium ion permeation are as low as about $10/\mu^2$ of membrane surface (Moore, Narahashi & Shaw, 1967; Hille, 1969). Taking this estimate at face value, each sodium channel would carry up to 1,000 Na ions per impulse. The movement of six or so charges per channel would produce a "gating current" that would be negligible by comparison.

5. The chemical nature of this formulation carries with it the assumption that all the structures described by the reaction are chemically identical. As long as the individual reactions are independent of each other, the possibility of heterogeneity arises only from the random distribution of thermal energy, which is described by Maxwell-Boltzmann statistics. However, it is also possible that the structures undergoing the individual reactions may be chemically different (e.g., have a different valency) or that the local membrane environment could vary. The effect of such heterogeneity could also be treated statistically.

6. The source of energy for the reactions is the membrane potential or, more correctly, whatever fraction of the total membrane potential is experienced by the movement of the charges in the channel structure. Two points are worth emphasizing here:

(a) In view of paragraph (4) above, the energy dissipation will be very small compared to that which is due to the ion current flows themselves.

(b) Since net movement of charged particles undergoing the reaction is thought to occur only during a change from one steady level of activation to another, the steady levels may correspond to true thermodynamic equilibria so far as the controlling reaction is concerned. No energy is dissipated by the reaction itself when the membrane structures are in a steady state. Of course energy will continue to be dissipated by the ion current flows controlled by the voltage-dependent reactions – the total system (voltage-dependent reactions + ionic current flows) cannot be in true equilibrium, except at one potential: the ionic equilibrium potential. However, no current is then recorded; therefore, this potential cannot be used directly to study the kinetics of the system.

Voltage Dependence of Equilibrium Constant

It is clear however that we may, thermodynamically speaking, "isolate" a voltage-dependent reaction for theoretical purposes and treat it in the standard chemical ways. Thus, the equilibrium constant of, for example, the s reaction, may be defined in the usual way (as the ratio of forward and

reverse reaction rates):

$$K_s = \alpha_s/\beta_s = s_\infty/(1-s_\infty). \tag{7}$$

As expected in a true equilibrium, the ratio of rates is also the ratio of the equilibrium "concentrations" in the two states. K_s may therefore be obtained from the experimental results by dividing the degree of activation s, as in Eq. (7), by its complement. This has been done and is plotted in Fig. 2 on semi-logarithmic coordinates. The points are well fitted by a straight line, indicating that the equilibrium constant is very steeply and exponentially related to membrane potential:

$$s_\infty/(1-s_\infty) = \exp[(E_m+77)/6] \tag{8}$$

and, therefore, s_∞ is described by the relation

$$s_\infty = 1/(1+\exp[(E_m+77)/6]) \tag{9}$$

where E_m is expressed in millivolts.

Eq. (9) has the same form as the equation used by Hodgkin and Huxley (1952, p. 501, Eq. 1) to fit h_∞, the sodium inactivation process:

$$h_\infty = 1/(1+\exp[(V_h-V)/7]) \tag{10}$$

which appears slightly different because of the way in which the membrane potential is defined. Weidmann (1955) has also fitted the sodium inactivation process in Purkinje fibers by a similar equation:

$$h_\infty = 1/(1+\exp[(V_h-V)/5]) \tag{11}$$

which is even steeper.

In fact, it appears that all the variables to which membrane currents are *directly* proportional (and which do not require a power function – *see* above) can be well described by this empirical form. However, n_∞ is not at all well described in this way, since it continues to increase slowly over tens of millivolts beyond the range of potentials at which it is steeply dependent on E_m. Perhaps this is another reason for suspecting that there might be a better formulation for those currents with a sigmoid time course of activation; we have not pursued this question, although Tille (1965) has presented an alternative approach which does not use power functions.

There is an analogy here between the voltage dependence of the equilibrium constant (e.g., K_s) and the voltage dependence of the ratio of ion fluxes

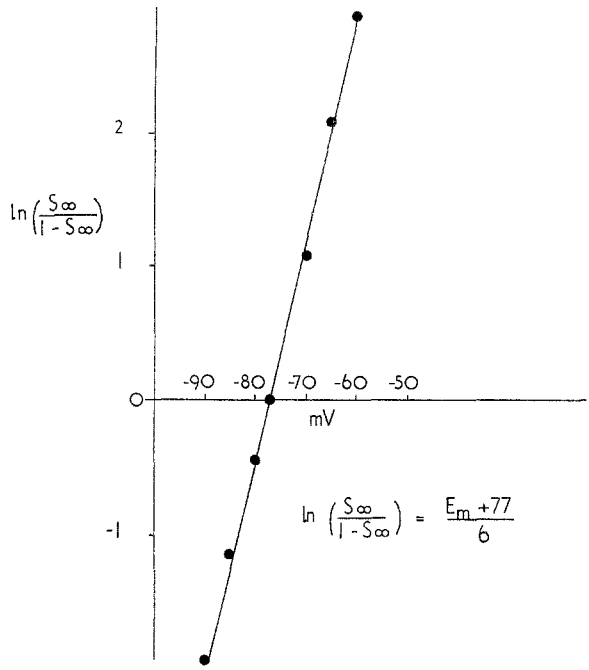


Fig. 2. Voltage dependence of equilibrium constant, $K_s = s_\infty/(1-s_\infty)$, plotted on semi-logarithmic scale. The relation is a steep linear relation fitted by Eq. (8)

across membranes, provided that the latter is derived using the independence principle (Ussing, 1949; Hodgkin & Huxley, 1952). If the influx and efflux processes are independent, then the flux ratio is given by

$$\frac{M_{\text{IN}}}{M_{\text{OUT}}} = \exp\left[\frac{z\,e}{kT}(E - E_{Eq})\right] \tag{12}$$

where z is the valency of the ion. This has the same form as Eq. (9) which might be rewritten

$$K_s = \frac{\alpha_s}{\beta_s} = \exp\left[\frac{z\,e\eta}{kT}(E_m + 77)\right] \tag{13}$$

where η is the fraction of the membrane potential which is experienced by the $a \rightarrow b$ transition. η will be less than unity if the charge movement is limited or if electrostatic shielding occurs. This equation embodies Hodgkin and Huxley's method for calculating z. Thus, from the correspondence of Eqs. (9) and (13), $\frac{ze\eta}{kT} = \frac{1}{6}$. Taking η as one (its highest possible value), and kT/e as 25 mV, the valency of the s particles is about four. Since no

power functions are involved, this is the minimum valency. The dependence of $(x_1)_\infty$ and $(x_2)_\infty$ on potential is less steep, and therefore the valencies are lower (around 1 to 2), or the reactions are more "shielded" from the electric field. The valency of the x_2 system in atrial muscle is also about two (Brown & Noble, 1969*b*).

Eq. (13) may also be obtained by treating the ratio $s_\infty/(1-s_\infty)$ as a Maxwell-Boltzmann distribution, and it may be noted that the slope of the ratio in semi-log coordinates (Fig. 2) should be inversely related to temperature. However, the predicted effect is small: the slope should increase by about 10% for 30 °C cooling. Although such small effects would be difficult to measure accurately, it would be of some interest if a sizeably larger effect were observed (*see* Dudel & Rüdel, 1969; *see* Conclusion).

Voltage Dependence of Rate Coefficients: Constant Field Approach

Whereas it is a relatively simple matter to treat the equilibrium state as a function of potential, it is a more difficult problem to treat the voltage dependence of the rate coefficients independently. Hodgkin and Huxley adopted, but did not rigidly keep to, an approach which has proved very useful in electrophysiology – even if only as a starting point. The approach assumes that within the membrane charged particles move under the influence of a constant electric field. As first applied to the voltage dependence of ion permeation (Goldman, 1943; Hodgkin & Katz, 1949), the theory predicted that the unidirectional flux of an ion should increase exponentially with potential when the driving force is small, but only linearly with larger driving forces. The slope of the linear portion is given by the product of the partition coefficient, the intra-membrane mobility, and the particle concentration on the side from which the flux originates.

If the mechanism of the *m*, *h* and *n* reactions is controlled by the movement of gating particles which are themselves charged, the reaction rates α and β should also be voltage-dependent in a similar way. Thus,

$$\alpha \propto \frac{V-V_0}{1-\exp\left[-(V-V_0)/k\right]}. \tag{14}$$

This equation provided a good description of α_m and α_n in squid and also of most of the rate coefficients in toad myelinated nerve.

In order to satisfy the requirement that the fraction of particles in a transition state should be small (*see* above), it must be assumed that the partition coefficient is very small; the partition coefficient can be thought of as a large energy barrier which ensures that the overall kinetics are first-

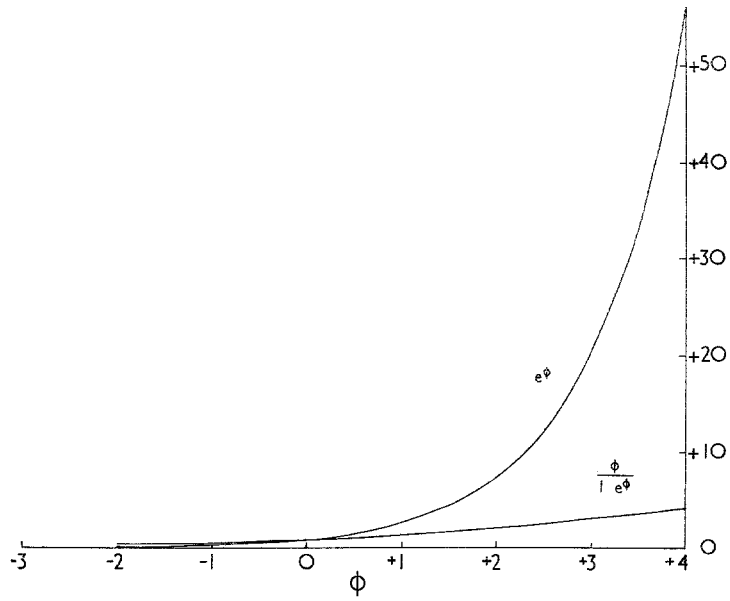


Fig. 3. Curves for the functions $\alpha = e^{\phi}$ and $\alpha = \phi/(1-e^{\phi})$, where ϕ is a linear function of the membrane potential. The first form is that given by a single energy barrier model (Eq. 23). The second form is that given by the constant field theory (Eq. 14)

order. Over the time scale required for redistribution to occur *within* the constant field phase, the quantities of gating particles in either the a or b states will be virtually constant. If this were not the case, the rate coefficients would necessarily depend on the history of the membrane potential and not solely on the "instantaneous" value of E_m (but *see* Frankenhaeuser, 1963, for a case in which this does not hold).

This approach is attractively simple and makes use of equations which are already familiar to membrane biophysicists. There are, however, some difficulties:

1. It seems unlikely that the electric field in the membrane (or membrane region over which the $a \rightarrow b$ transition occurs) should be constant, since a large amount of charge must be transferred and this would require a large degree of "shielding" to keep the field uniform.

2. Although the theory works well for α_m and α_n, it does not fit some of the other rate coefficients. Thus, in squid, β_n increases much too slowly and β_m too quickly with hyperpolarization to allow a fit by the appropriate constant field equations. Both of these coefficients were better fitted by simple exponentials, as was α_h. β_h presents a further difficulty in that it levels off as the membrane is depolarized so that the function is sigmoid.

Since their immediate aim was to reproduce the potentials changes in nerve rather than to produce a complete physico-chemical theory, Hodgkin and Huxley avoided the problem of "explaining" these deviations and used whatever functions were convenient. Moreover, in view of these empirical variations in the rate coefficient functions, any theoretical approach is faced with the same difficulty. The best we can hope for, in the absence of detailed knowledge of the molecular structures involved, is a relatively simple basic theory with plausible explanations for the observed deviations.

Unfortunately, the constant field theory is not sufficiently general to easily allow modifications to deal with the observed deviations. It seems worthwhile, therefore, to outline an alternative approach which is more general than the constant field theory.

Voltage Dependence of Rate Coefficents: Transition State Theory Approach

Any physical model for the permeability reaction must deal with the extreme slowness of the rate coefficients α and β. This is obviously true in the case of cardiac current kinetics, but it is also true in nerve. The millisecond time scale is still quite slow compared to the rate at which ions may cross the membrane. Thus, as many as several hundred ions may move via a single channel in the average time for which it is conducting (*see* above).

The constant field formulation of the rate coefficients deals with their slowness by assuming a large energy of activation (corresponding to the low partition coefficient) at either side of the constant field phase. Both of the energy barriers are treated as potential-independent. The membrane potential only influences the movement of charged gating particles in the constant field phase. Thus, if the constant field phase is represented as a series of small energy barriers the membrane potential influences their magnitudes as in Fig. 4.

In the absence of more detailed knowledge of the microchemistry of the membrane, it is impossible to say if the constant field theory is realistic. The fact that some of the rate coefficients are poorly fitted by the constant field equations suggests that in some cases, at least, it is not a good approximation, and that a more general treatment is required.

Despite our ignorance of the mechanism of the reaction, it may still be helpful to have some physical interpretations in mind when applying transition state theory. There are several possibilities:

1. Hodgkin and Huxley suggested that charged "gating" particles move under the influence of the local electric field, blocking the movement of ions

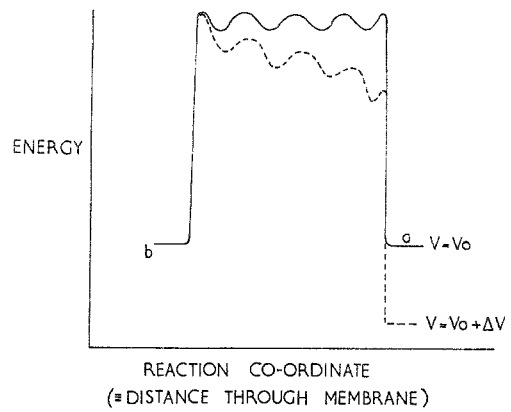


Fig. 4. Energy profile assumed by constant field theory. The large energy jumps at each "edge" of the "membrane" correspond to the partition coefficient, β. The smaller jumps are those due to electrodiffusion. For the sake of simplicity, these small jumps are neglected individually, and their overall effect on rate of movement is included in the mobility term, u

when in the b position. The energy profile could, of course, be grossly different from that predicted by the constant field theory.

2. A specialized membrane structure might undergo a conformational change corresponding to the permeability reaction. Various dipolar groups in phospholipid (Goldman, 1965) or protein molecules (Tobias, 1964) might serve such a role. On the other hand, the structural change might involve much larger molecules, incorporating sufficient *net* dipole moment to give the reaction its steep voltage dependence. The isolation of large enzyme molecules underlying active transport in bacterial membranes (*see* Pardee, 1968, for review) makes a high degree of molecular specialization for ionic channels seem more plausible.

3. Electron or proton transfer reactions might form an essential step leading to a subsequent conformational change in the membrane structure. In this case, the voltage dependence will be determined by the number of charge transfer reactions that are required. One of the advantages of a model of this kind is that, at extremes of potential where the charge transfer rate becomes large, the subsequent conformational change (if relatively insensitive to the electric field) could itself become rate limiting. This would give rise to sigmoid rate coefficient functions of the kind sometimes observed (e.g., β_h in squid nerve, α_{x_2} in Purkinje fibers).

At present, therefore, the only features of the mechanism which can be regarded as certain are that the structures undergoing the reaction are charged and that the energy barriers encountered during the reaction are

large enough to give rate coefficients as small as those observed experimentally. A more general way of treating this situation is to use transition state theory to obtain expressions for the rate at which the reaction proceeds over one or more energy barriers. This theory relates the rates to the energies required to form transition (or "excited") states at the peaks of the energy barriers. The nature of the rate coefficients therefore differs from that predicted by the constant field theory. In particular, whereas the concept of "partition" coefficients and permeability are restricted to the description of the movement of particles, the term "transition state" may equally well be thought of as a structural change. Thus, the reaction coordinate for the reaction need not correspond to a spatial coordinate.

Since our purpose is mainly to illustrate the approach, we shall simplify the problem by assuming that there is only one energy barrier involved in the reaction. It will be shown that this assumption leads to rate coefficients which are simple exponential functions of the membrane potential. Other rate coefficient functions, such as the linear exponential form and the sigmoid form, may be obtained by using multiple energy barrier models (*see* Woodbury et al., 1968; *see* Conclusion).

In the case of a single energy barrier, the potential energy is assumed to rise as the reaction proceeds until it reaches a maximum value corresponding to the transition state $\pm$. The potential energy then falls as the a state is formed. The energy required to form the transition state is the activation energy for the reaction. The continuous curve in Fig. 5 represents the energy profile when the electric field is such that the energies of the a and b states are equal. The interrupted line represents the profile when a field is applied which favors the formation of the a state. The value of the transmembrane potential when the energies of the a and b states are equal will be referred to as V_0. In general, V_0 is not zero (e.g., V_0 for the s reaction is about -75 mV). This may mean either that the electrical field across the membrane may be zero at a non-zero potential difference [as would happen if surface membrane charges greatly influence the membrane field – *see* Chandler, Hodgkin and Meves (1965); Rojas (1968); Ehrenstein and Gilbert (1969)] or that the system is not chemically symmetric in the sense that the energies of the a and b states may be unequal even when the membrane electrical field is zero.

Let:

z = the apparent valency of the structure undergoing conformational change. This may not be the true valency since some or all of the charge may move through a potential which is smaller than the total membrane potential (i.e., η may be less than 1).

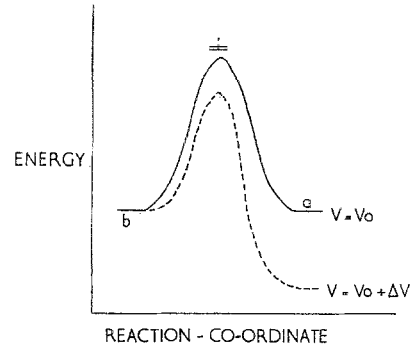


Fig. 5. Energy profiles assumed in transition state theory when only one energy barrier is rate-limiting. The continuous line represents the energy profile when the energies of the a and b states are equal (i.e., at V_0). The interrupted line represents the effect of adding a potential, ΔV

γ = fraction of the total membrane potential that affects the $a \rightarrow \pm$ transition.

N = Avogadro's number.

e = unit charge (1.6×10^{-19} coul).

$\Delta V = V - V_0$

$G(a)$, $G(b)$, $G(\pm)$: be the free energy per mole of the states a, b, and $\pm$;

$H(a)$, $H(b)$, $H(\pm)$: be the heat energy per mole of the states a, b, and $\pm$;

$S(a)$, $S(b)$, $S(\pm)$: be the entropy per mole of the states a, b, and $\pm$.

where $\Delta G = \Delta H - T\Delta S$ relates the *differences* in the various energies. The equilibrium populations in the a and b states will be determined only by the free energy difference between the a and b states, $G_{ab} = G(a) - G(b)$. As noted already above, the ratio of populations will be given by a Maxwell-Boltzmann distribution:

$$\frac{p(a)}{p(b)} = \frac{s_\infty}{1 - s_\infty} = \exp[-G_{ab}/RT]. \tag{15}$$

By assuming that G_{ab} is linearly related to the membrane potential,

$$\Delta G_{ab} = z e N \Delta V \tag{16}$$

we obtain Eq. (10) once again (*see* Voltage Dependence of Equilibrium Constant). This relation was used to estimate the valency z from the empirically determined $s_\infty(E_m)$ relation. We may now extend the treatment to

derive the voltage dependence of the rate coefficients, α and β, by making some assumptions about the transition process. The treatment follows a theory developed by Eyring and coworkers which attempts to predict absolute reaction rates from the relative energies of the initial and transition states. (*See* Glasstone, Laidler & Eyring, 1941, or Frost & Pearson, 1963, for a general account of the theory.)

The important assumption of the transition state theory is that, up to the formation of the transition state, the population of various energy levels is in equilibrium with the initial state. The concentration of structures in the transition state is therefore related, using a Maxwell-Boltzmann term, to the energy of the $\pm$ state. Thus, for the reaction $b \to a$ we have: $b \leftrightarrow \overrightarrow{\pm} \to a$; and for $a \to b$, $b \leftarrow \overleftarrow{\pm} \leftrightarrow a$, where $\overrightarrow{\pm}$ stands for transition state structures moving from b to a, and $\overleftarrow{\pm}$ stands for transition state structures moving from a to b. The double-headed arrows ($\leftrightarrow$) indicate the existence of equilibria up to the formation of $\pm$. The single arrows indicate the decomposition of $\pm$ into the final state.

According to the theory, the frequency of decomposition of the excited state is given by a frequency term, $\kappa KT/h$ ($=KRT/Nh$). Apart from the factor κ, the frequency term is the same for all reactions: $\sim 10^{13}$ sec^{-1}. The transmission coefficient κ is the probability that the reaction will go forward once the transition state is formed. For simple energy barriers (as assumed in Fig. 5), this factor is simply one. The rate of the reaction is then directly proportional to the fraction of systems in the excited state. Writing equations for the s system once again,

$$\alpha_s = (kT/h) \exp[-\Delta G_{\pm b}/RT], \tag{17}$$

$$\beta_s = (kT/h) \exp[-\Delta G_{\pm a}/RT]. \tag{18}$$

Further analysis is possible if the temperature dependence is known, since ΔG may then be separated into enthalpy and entropy components, $\Delta G = \Delta H - T\Delta S$. Eqs. (17) and (18) then become

$$\alpha_s = (kT/h) \exp[\Delta S_{\pm b}/R] \exp[-\Delta H_{\pm b}/RT], \tag{19}$$

$$\beta_s = (kT/h) \exp[\Delta S_{\pm a}/R] \exp[-\Delta H_{\pm a}/RT]. \tag{20}$$

The membrane potential may now be included by assuming that the free energy changes are linearly related to the membrane potential so that (*see*

Glasstone et al., 1941, p. 576)

$$\Delta G_{\pm a}=(\Delta G_{\pm a})_0+\gamma z e N \Delta V, \tag{21}$$

$$\Delta G_{\pm b}=(\Delta G_{\pm b})_0-(1-\gamma) z e N \Delta V \tag{22}$$

where $(\Delta G_{\pm a})_0$ and $(\Delta G_{\pm b})_0$ are the values when $\Delta V=0$, i.e., when $\alpha_s=\beta_s$. γ is the fraction of the membrane potential involved in the $a \rightarrow \pm$ transition. Eqs. (17) and (18) may now be written as

$$\alpha_s=(kT/h)\exp[(\Delta S_{\pm b})_0/R]\exp[-(\Delta H_{\pm b})_0/RT] \cdot \exp[(1-\gamma) z e N \Delta V/RT], \tag{23}$$

$$\beta_s=(kT/h)\exp[(\Delta S_{\pm a})_0/R]\exp[-(\Delta H_{\pm a})_0/RT] \cdot \exp[-\gamma z e N \Delta V/RT] \tag{24}$$

which gives α_s and β_s in terms of the energy changes involved in the formation of $\overrightarrow{\pm}$ and $\overleftarrow{\pm}$ (*see* Vetter, 1967, p. 140).

We will now discuss some of the consequences and implications of this treatment.

Symmetry of Reaction

Unless $\gamma=0.5$, α and β will not be symmetrical about V_0. In the case of the s reaction, the rate coefficients α_s and β_s are, in fact, fairly symmetric over the voltage range investigated (although the range is rather more limited in the case of β_s). However, some of the nerve kinetics and also the x_2 kinetics in cardiac membranes show a considerable degree of asymmetry. In microscopic terms, the variations between current components could be attributed to different membrane potential profiles, or to more fundamental variations in the chemical nature of the $\pm$ state in relation to a or b.

Activation Enthalpy and Entropy

In this section we shall use the single-barrier equations to obtain estimates of the ΔH and ΔS of activation. Since the results would be numerically different if a multi-barrier model were used, we shall refer to the calculated values as "apparent" energies of activation. The extent to which the general conclusions may be modified by the use of multi-barrier models will be discussed later (*see* Conclusion).

It may be helpful to outline a simple expectation which, if correct, would enable the results to be given some kind of order. It is clear from Eqs. (17) and (18) that as α or β is made smaller, so the apparent free energy of activation must become larger. If *all* of the change in free energy of activation were attributable to a change in ΔH, it would be possible to predict how

much greater the temperature dependence of the reaction should be when the rate of the reaction is slower. This prediction could then be tested empirically. At present, there are two ways of obtaining information on reactions occurring at different rates. First, the rate for a particular reaction varies with voltage so that predictions can be made concerning the voltage dependence of the Q_{10} of the reaction. Second, different reactions occur at different rates so that we may also compare the values of ΔH for different reactions. We will discuss each of these cases in turn.

Variation of Temperature-Dependence with Voltage. For the single-barrier model, the relation between $Q_{\Delta T}$ and ΔH may be obtained as follows:

$$(Q_{\Delta T})_\alpha = \frac{\alpha_{(T_0+\Delta T)}}{\alpha_{T_0}} = \frac{T_0+\Delta T}{T_0} \exp\left[\frac{-\Delta H_{\pm b}}{R}\left(\frac{1}{T_0+\Delta T}-\frac{1}{T_0}\right)\right] = \frac{T_0+\Delta T}{T_0} \exp\left[\frac{\Delta H_{\pm b}}{RT_0}\,\frac{\Delta T}{T_0+\Delta T}\right], \tag{25}$$

$$\frac{\Delta H_{\pm b}}{RT_0} = \frac{T_0+\Delta T}{\Delta T} \log_e\left[\frac{T_0}{T_0+\Delta T}(Q_{\Delta T})_\alpha\right] = \frac{T_0}{\Delta T}\log_e(Q_{\Delta T})_\alpha. \tag{26}$$

The temperature dependence of a rate coefficient can thereby be interpreted in terms of an activation enthalpy. In the case of the s reaction, the Q_{10} at V_0 is 6 (Noble & Tsien, 1968). Thus,

$$(\Delta H_{\pm b})_0 = \frac{310}{10}\log_e\left(\frac{300}{310}\cdot 6\right) RT_0 = 54.5\,RT_0 = 33 \text{ kcal/mole}.$$

Other known rate coefficients are considerably less temperature-dependent than α_s and therefore correspond to smaller activation enthalpies, ranging down to an apparent enthalpy of 8,400 kcal/mole in the case of the β_m reaction in myelinated nerve (*see* Table).

Most of the empirical measurements of the temperature dependence of rate coefficients have been confined to a single value of potential, for obvious reasons. The evaluation of enthalpy is, of course, appropriate to that potential only. Over a range of potentials, the voltage dependence of the rate should also be reflected as a voltage dependence of $Q_{\Delta T}$. The faster the rate, the smaller the activation enthalpy, and, therefore, the smaller the variation of rate with temperature.

The influence of voltage on $Q_{\Delta T}$ can be predicted quantitatively if the process is well described by a single barrier model. For example, a 20-fold increase in rate should decrease the Q_{10} by only 10%. Even with larger variations, it is unlikely that experimental limitations on potential and temperature will allow a useful assessment of enthalpy by the measurement

of $Q_{\Delta T}$. The prediction would be more useful if, in fact, experimental $Q_{\Delta T}$'s *exceeded* that predicted theoretically. If the nonexponential rate coefficients (and particularly those which saturate at extremes of potential) reveal the importance of other processes which can become rate-limiting, one would expect that some evidence for this might arise from a detailed study of temperature dependence. Dudel and Rüdel (1969) have observed a large decrease in the steepness of the $h_\infty(E_m)$ relation in Purkinje fibers on cooling, although it remains to be seen if the form of the rate coefficients also changes markedly with temperature.

Activation Energies for Different Reactions. Two of the most striking properties of the reactions controlling ion current flow are the wide variation in the *absolute* magnitude of the rate coefficients (time constants range from a fraction of a millisecond up to several seconds) and the wide variation in their temperature dependence (*see* Table). In a general way, the prediction referred to earlier is correct: the Q_{10} is larger as the absolute rate becomes smaller. However, a quantitative comparison between the apparent free energy and the apparent enthalpy suggests that entropy changes may play a different role in various permeability reactions. (*See* Johnson, Eyring & Polissar, p. 21 and chapter 8 for a discussion of entropy changes in other biological reactions.) Using α_s as an example once again, the apparent entropy may be calculated as follows (Eq. 17 given as before):

$$\alpha_s=(kT/h)\exp[-\Delta G_{\pm b}/RT] \quad \text{repeat of (17)}$$

since $(\alpha_s)_{V_0}=0.5\ \text{sec}^{-1}$, and $kT/h=10^{13}\ \text{sec}^{-1}$,

$$(\Delta G_{\pm b})_{V_0}/RT=30.7$$

and since $(\Delta H_{\pm b})_{V_0}/RT=54.5$ (*see* above)

$$(\Delta S_{\pm b})_{V_0}=23.8\,R=48\ \text{cal/°C}.$$

For the purpose of illustrating the range of apparent energies of various rate coefficients, we have largely relied upon Frankenhaeuser and Moore's (1963) characterization of the ionic currents in the node of Ranvier of toad. The kinetic variables m, n and h are remarkably similar to those of squid, with the notable exceptions of α_m and β_m which are considerably less temperature-dependent in toad nerve than in squid [where they have a more typical Q_{10} of 3 (Moore, 1958)].

In all of the reactions listed in the Table, with the exception of α_m and β_m, the apparent entropy is positive, indicating that the transition state is more

Table. *Apparent energy values of various Hodgkin-Huxley rate variables, using the single energy barrier model*

Tissue	Rate variable	Rate value [sec^{-1}]	Apparent $\Delta G/RT$	Q_{10}	Apparent $\Delta H/RT$	Apparent $\Delta S/R$
Toad	α_m	10^4	20.8	1.84	18.5	−2.3
Nerve	β_m	7.5×10^3	21.1	1.68	14.1	−7.0
	α_h	1.3×10^3	22.8	2.80	29.1	6.3
	β_h	1.7×10^3	23.6	2.93	30.5	6.9
	α_n	1.7×10^3	23.6	3.20	33.1	9.5
	β_n	1.2×10^3	22.9	2.76	28.8	5.9
Purkinje fiber	α_s	0.5	30.7	6	54.5	22.8

disordered than either the *a* or *b* state. However, it may now be seen that there is no quantitative order in the values for ΔH. ΔS, although usually positive, varies greatly, so that the variation in absolute rate is not solely attributable to variations in ΔH. In fact, it is clear that, unless the entropy of activation were positive, the slower reactions (*n* and *s*) would occur very much more slowly than they do. A possible interpretation of the positive entropy of activation will be discussed later (*see* Conclusion).

More recent studies of squid axon membrane have revealed current changes which are even slower than the cardiac reactions we have described. The inactivation of the outward *K* current (Ehrenstein & Gilbert, 1965) and the full reavailability of the Na current (Adelman & Palti, 1969) occur over a time scale of 10 to 100 sec. Although the functional significance of these reactions is unknown, it would be useful to know their temperature dependence and the relative contributions of enthalpy and entropy to the total activation energy. Unless the squid reactions are exceedingly temperature-dependent, the apparent entropy change will be even larger than for the *s* reaction.

Conclusion

General Conclusions Concerning Single Barrier Results

The discovery that the mechanisms controlling the outward currents in cardiac membranes obey first-order kinetics with rate coefficients which are approximately exponential functions of membrane potential has encouraged us to treat the membrane reactions underlying these mechanisms in the simplest possible way, i.e., as chemical transitions with one rate-limiting "excited" state. Using Eyring's rate theory, we have obtained estimates of the energy changes required to form the excited states, in the case of the slow K current reaction in cardiac muscle, as well as the faster Na and K

current reactions in nerve. In most cases, the ΔH of activation is larger than the ΔG of activation; the entropy change in forming the activated state must *favor* the reaction. The exception in these results appears to be the fastest reaction, m, in toad nerve. However, it is possible that the entropy of activation may also be positive in the case of the m reaction, particularly if more than one energy barrier is involved (*see* below).

It must be emphasized however, that these results should be treated with some caution. First, not all of the rate coefficients are simple exponential functions of voltage, so that in some cases a more complex energy profile is required. Second, in order to obtain values for ΔS and ΔH of activation, it must be assumed that temperature has no effect on the relative energies of the a, b and $\pm$ state, but that it simply changes the probability that an individual structure will undergo a transition. This assumption would be invalid if temperature changes induce changes in membrane structure which in turn alter the energy levels of the permeability reactions. Until more is known of the structure and chemistry of excitable membranes, it may be premature to carry this theoretical approach further. However, the analysis does suggest that it may be worthwhile to investigate the effects of temperature in more detail. Although the theory predicts that the shape of the rate coefficients should change, the predicted effects are relatively small. If temperature changes were also to influence the energy levels, as suggested above, more marked effects on the rate coefficients and steady-state activation relations might be observed experimentally (*see* Dudel & Rüdel, 1969).

Multi-Barrier Models

The assumption that only one excited state is involved in the membrane permeability reactions is obviously an oversimplification. However, the approach we have described may be extended to reactions with more complex energy profiles. Woodbury et al. (1968) have developed a model for ion permeation which applies Eyring rate theory to a four-barrier energy profile. Similar analysis could be applied to the reactions controlling membrane permeability. In particular, it is possible with a number of barriers to derive voltage-dependent rate coefficients which are not simple exponentials; under suitable conditions (*see* Woodbury et al., 1968; Woodbury, 1969), these may approximate the linear-exponential forms that arise from the constant field treatment.

Simple exponential coefficients might then be regarded as a special case where one of the energy barriers is much higher than the others. On the other hand, the sigmoid voltage-dependence of certain rate coefficients (β_h in squid nerve, α_{x_2} in Purkinje fiber, β_{x_2} in atrial muscle) might be

attributed to an energy barrier which is relatively insensitive to voltage (e.g., an electrically neutral conformational change) which could become rate-limiting.

The advantage of the transition state approach over the constant field formulation is that these variations may all be included in a fairly general framework. But it is important to note that the transition state theory does not, by itself, provide a physical interpretation for individual rate coefficients – a large number of different physical mechanisms might lead to the same energy profile. In the present state of knowledge of the membrane, it seems more reasonable to use a fairly general approach which is not committed to a particular physical model.

Significance of Activation Entropy

The conclusion that the entropy of activation of the reactions controlling the ionic currents is usually positive is in striking contrast to the large negative entropy of activation for the ion transport process itself. Like the rate coefficients for the controlling reactions, the rate coefficients for ion transfer are also small. In these cases, however, the Q_{10} is usually much smaller than that of the controlling reactions. Thus, the Q_{10} of membrane conductance is usually about 1.3, whereas the Q_{10} for the controlling reactions lie between 2 and 6. One factor which is probably largely responsible for the negative entropy of activation in the case of ion transfer is that the sites available for ion transport are very sparsely distributed (Hille, 1969) so that only ions colliding with the membrane at certain points and, perhaps, in certain directions may cross. The ions crossing the membrane would then be more ordered than those in free solution. On the other hand, the positive entropy of activation of the controlling reactions requires that in these cases the transition states should be less ordered than the final and initial states. The most likely mechanism for this in aqueous systems is a decrease in the ordering of water molecules (*see* Gill, 1965). Thus, if during the formation of the transition state, charges of opposite signs are brought closer together, then fewer water molecules would be ordered in the less intense electric fields. In this context it would be interesting to know if the structures responsible for the controlling reactions are significantly hydrated.

Finally, these energetic differences lend further support to the view that the Hodgkin-Huxley variables (or their equivalents in other mathematical formulations) correspond to real reactions which are separate from the processes immediately responsible for ion transport.

Further advances in studying these reactions will require suitable "probes" of the membrane structure which may detect changes obeying the kinetics

described by the voltage clamp work. Hopeful beginnings on this problem have been made using light scattering and birefringence (Cohen, Keynes & Hille, 1968) and fluorescence measurements (Tasaki, Watanabe, Sandlin & Carnay, 1969), but although changes accompanying excitation have been detected, it is not clear whether the optical effects correspond directly to any of the Hodgkin-Huxley variables.

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References

Adelman, W. J., Jr., and Y. Palti. 1969. Relation between (K_0) and long duration voltage conditioning in determining inactivation levels in squid giant axon. *Biophys. J.* **9**:SAM H-4.

Adrian, R. H. 1969. Rectification in muscle membrane. *Progr. Biophys.* **19**:341.

Agin, D., and E. Rojas. 1963. A third order system for the squid giant axon membrane. *Proc. Conf. Eng. Med. Biol.* **5**:4.

Brown, H. F., and S. J. Noble. 1969*a*. Membrane currents underlying delayed rectification and pacemaker activity in frog atrium. *J. Physiol.* (*in press.*)

— — 1969*b*. A quantitative analysis of the slow component of delayed rectification in frog atrium. *J. Physiol.* (*in press*).

Chandler, W. K., A. L. Hodgkin, and H. Meves. 1965. The effect of changing the internal solution on sodium inactivation and related phenomena in giant axons. *J. Physiol.* **180**:821.

—, and H. Meves. 1965. Voltage clamp experiments on internally perfused giant axons. *J. Physiol.* **180**:788.

Cohen, L. B., R. D. Keynes, and B. Hille. 1968. Light scattering and birefringence changes during nerve activity. *Nature* **218**:438.

Cole, K. S. 1968. Membranes, ions and impulses, Univ. Calif. Press, Berkeley and Los Angeles.

—, and J. W. Moore. 1960. Potassium ion current in the squid giant axon: dynamic characteristics. *Biophys. J.* **1**:1.

Ehrenstein, G., and D. L. Gilbert. 1966. Slow changes in potassium permeability in aquid giant axon. *Biophys. J.* **6**:553.

Eyring, H. 1935. The activated complex in chemical reactions. *J. Chem. Phys.* **3**:107.

FitzHugh, R. 1965. A kinetic model of the conductance changes in nerve membrane. *J. Cell. Comp. Physiol.* **66, Suppl. 2**:111.

Frankenhaeuser, B. 1963. A quantitative description of potassium currents in myelinated nerve fibres of *Xenopus laevis*. *J. Physiol.* **169**:424.

—, and L. E. Moore. 1963. The effect of temperature on the sodium and potassium permeability changes in myelinated nerve fibres of *Xenopus laevis*, *J. Physiol.* **169**:431.

Frost, A., and R. Pearson. 1963. Kinetics and Mechanism. Wiley, New York.

Gill, E. W., 1965. Drug receptor interactions. *Prog. Med. Chem.* **4**:39.

Glasstone, S., K. J. Laidler, and H. Eyring. 1941. The Theory of Rate Processes. McGraw-Hill, New York.

Goldman, D. E. 1943. Potential, impedance and rectification in membranes. *J. Gen. Physiol.* **27**:37.

Goldman, D. E. 1964. A molecular structural basis for the excitation properties of axons. *Biophys. J.* **4**:167.

Hauswirth, O., D. Noble, and R. W. Tsien. 1968. Adrenaline: mechanism of action on the pacemaker potential in cardiac Purkinje fibres. *Science* **162**:916.

— — — 1969. The mechanism of oscillatory activity at low membrane potentials in cardiac Purkinje fibres. *J. Physiol.* **200**:255.

Hill, T. L. 1967. Electric fields and the cooperativity of biological membranes. *Proc. Nat. Acad. Sci.* **58**:111.

Hille, B. 1969. Ionic channels in nerve membranes. *Progr. Biophys.* **21** (*in press*).

Hodgkin, A. L., and A. F. Huxley. 1952. A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol.* **117**:500.

—, and B. Katz. 1949. The effect of sodium ions on the electrical activity of the giant axon of the squid. *J. Physiol.* **108**:37.

Hoyt, R. C. 1963. The squid giant axon: Mathematical models. *Biophys. J.* **3**:399.

— 1968. Sodium inactivation in nerve fibres. *Biophys. J.* **8**:1074.

Laidler, K. J. 1965. Chemical kinetics. McGraw-Hill, New York.

Moore, J. W. 1958. Temperature and drug effects on squid axon membrane ion conductances. *Fed. Proc.* **17**:113.

— T. Narahashi, and T. I. Shaw. 1967. An upper limit to the number of sodium channels in nerve membrane? *J. Physiol.* **188**:99.

Mullins, L. J. 1968. A single channel or a dual channel mechanism for nerve excitation? *J. Gen. Physiol.* **52**:550.

Noble, D. 1965. Electrical properties of cardiac muscle attributable to inward-going (anomolous) rectification. *J. Cell. Comp. Physiol.* **66, Suppl. 2**:127.

— 1966. Applications of Hodgkin-Huxley equations to excitable tissues. *Physiol. Rev.* **46**:1.

—, and R. W. Tsien. 1968. The kinetics and rectifier properties of the slow potassium current in cardiac Purkinje fibres. *J. Physiol.* **165**:185.

— — 1969*a*. Outward membrane currents activated in the plateau range of potentials in cardiac Purkinje fibres. *J. Physiol.* **200**:205.

— — 1969*b*. Reconstruction of the repolarization process in cardiac Purkinje fibres based on voltage clamp measurements of the membrane current. *J. Physiol.* **200**:233.

Pardee, A. B. 1968. Membrane transport proteins. *Science* **162**:632.

Tasaki, I., A. Watanabe, R. Sandlin, and L. Carnay. 1968. Changes in fluorescence, turbidity and birefringence associated with nerve excitation. *Proc. Nat. Acad. Sci.* **61**:883.

Tille, J. 1965. A new interpretation of the dynamic changes of the potassium conductance in the squid giant axon. *Biophys. J.* **5**:163.

Ussing, H. 1949. The distinction by means of tracers between active transport and diffusion. *Acta Physiol. Scand.* **19**:43.

Verveen, A. A., and H. E. Derksen. 1968. Fluctuation phenomena in nerve membrane. *Proc. I.E.E.E.* **56**:906.

Vetter, K. J. 1967. Electrochemical kinetics. Academic Press, New York.

Weidmann, S. 1955. The effect of the cardiac membrane potential on the rapid avaliability of the sodium-carrying system. *J. Physiol.* **127**:213.

Woodbury, J. W. 1969. Linear current-voltage relation for Na^+ channel from Eyring rate theory. *Biophys. J.* **9**:A.

— S. H. White, M. C. Mackey, W. L. Hardy, and D. B. Chang. 1968. Bioelectrochemistry, University of Washington Press, Seattle.