ENERGY-TRANSDUCING REACTIONS IN BIOLOGICAL MEMBRANES

II.* A MOLECULAR MECHANISM FOR THE PERMEABILITY CHANGES IN NERVE DURING THE PASSAGE OF AN ACTION POTENTIAL

By D. E. Weiss†

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Summary

A molecular model is proposed for the permeability changes associated with an action potential. The model membrane contains molecular “springs” composed of helical lipopeptides containing four to six consecutive phospholipid residues. The lipids form bilayers which bind the springs to the surrounding membrane and possess elastic contractile properties. In an expanded configuration, the springs prefer hydrated Na⁺ to K⁺; in a contracted configuration unhydrated K⁺ is preferred to Na⁺ and is solvated by oxygen components of the spring. Expansion or contraction depends on the ionic strength and composition of the solution in contact with the springs.

In the resting state, the springs contain mainly K⁺ and an outer layer of chelated Ca²⁺ or Mg²⁺ (M²⁺) which prevents reaction with outer Na⁺. Excitation displaces M²⁺ and causes the springs to expand more rapidly than they can close by the advancement of K⁺ under the exciting potential. The Na⁺ permeability rises rapidly as Na⁺ contacts the springs, which expand fully, and Na⁺ passes across the membrane because of the favourable electrochemical gradient which has been established by the sodium pump. M²⁺ accumulates in the boundary layer as it enters with the inflowing current but is left behind as Na⁺ passes through the membrane more rapidly than M²⁺. The inward Na⁺ currents are accompanied by outward K⁺ currents through neighbouring contracted sites so that K⁺ tends to accumulate in the outer boundary layer. The accumulatim M²⁺ and K⁺ eventually contract the springs and enable K⁺ to move through the membrane and to displace M²⁺ without opening of the springs. The membrane now has its maximum permeability to K⁺.

Local circuits are established between adjacent depolarized and polarized regions of the membrane and result in the propagation of the action potential. As the outflowing current of K⁺ recedes at sites behind the advancing spike, the membrane reverts to its original state.

Anaesthetics stabilize the K⁺ configuration by preventing expansion of the springs, either by crosslinking the springs or by increasing the elasticity and restoring force of the lipid. DDT has a converse effect by being adsorbed between expanded springs and inhibiting their closure.

I. INTRODUCTION

This, the first biological paper of the series, will discuss action potential phenomena, because, although complex, it is nevertheless the simplest of the biological energy-transduction reactions in biological membranes. Later papers will discuss other phenomena in order of increasing complexity. In an action potential, the

† Division of Applied Chemistry, CSIRO Chemical Research Laboratories, P.O. Box 4331, Melbourne, Vic. 3001.

energy of ion concentration gradients is converted into an ionic electrical current as a result of a change in membrane ion selectivity. In recent years several mathematical models, notably that of Hodgkin and Huxley (see Hodgkin 1964; Noble 1966), have described the action potential in detail, but these models do not propose a mechanism for the observed permeability changes. A number of theories of a more general nature have been offered (Karreman and Landahl 1953; Tobias 1958; Mullins 1959; Goldman 1962; Ehrenpreis 1964; Goldman 1964; Mueller and Rudin 1968; Tasaki 1968) in an attempt to provide a molecular explanation.

It is the purpose of this paper to show that action potentials can be accounted for as an ion-exchange phenomenon in which an excitatory current induces transient dilation of an ion-exchange polymer where the resultant conformational change alters ion selectivity, and stretches membrane bilipids whose rubber-like contractile properties participate in restoring the membrane to its original state. Drugs are postulated to alter action potentials by changing membrane elasticity. The dilation–contractile principles on which the model is based have been described in Part I of this series (Weiss 1969a). The model has been described in a preliminary note (Weiss 1969b).

II. THE “SPRING” CONCEPT

It is proposed in the model that there is a phospholipoprotein, or a related structure, which creates localized ion-exchange sites in the membrane where cations can cross; in other areas the membrane is impervious to cations. The phospholipoprotein assumes a helical, polypeptide configuration from which the lipids radiate. Van der Waal interactions between these lipid hydrocarbons and adjacent supporting hydrocarbon components of the membrane form bilayers with rubber-like properties (see Part I) and so form a molecular spring. Ionization of the phosphate groups creates electrostatic repulsive forces between adjacent charges which, together with osmosis, tend to expand the spring to an extent depending on the degree of interaction between the cations of the solution and the exchange sites.

In the expanded state, the size of the cavities containing the exchange sites, and the charge density of the latter, are such that of the alkali metals only hydrated Na+ (or Li+) can be accommodated most readily (see Part I). The cavity can, however, accommodate certain other hydrophilic cations, such as guanidinium or hydrazinium ions, instead of Na+. Dilation extends the attached bilayers into a more ordered structure where free rotation of hydrocarbon segments is hindered so that an opposing tension develops, as in stretched rubber. Dilation becomes more difficult the greater the tension in the spring.

In its contracted form, the spring can accommodate unhydrated K+, or unhydrated Rb+ and Cs+, but not the hydrated ions (see Part I). K+ can be so accommodated in preference to Na+ because of its lower hydration energy. In such a situation, the strong electrostatic interactions promote extensive ion association and reduce electrostatic repulsive forces so that the presence of K+ (or Cs+ or Rb+) favours contraction. The non-aqueous environment also reduces interaction with water and osmosis. Since the postulated interactions between the partial negative charges of the solvating components of the spring and the K+ ion are purely electrostatic, and do not involve a transfer of charge between the reactants, a highly specific spatial alignment is not necessary.
It is therefore concluded that the postulated phospholipid spring provides a reasonable mechanism for an ion-selectivity change as its properties are consistent with the known behaviour of simpler systems, but that, although such a phospholipopeptide is present in nervous tissue, there is no experimental evidence that it is associated with cation transport in membranes, although there is recent evidence that it is probably present in membranes (Trevor and Rodnight 1965). Possibly related phosphoproteins have been observed also in liver slices (Ahmed and Judah 1962) and in human erythrocytes (Judah, Ahmed, and McLean 1962).

III. Ion-exchange Equilibria

An assembly of springs is postulated within the model membrane to form an ion-exchange system. It should be noted that the ion selectivity of an ion-exchange resin is not constant, but varies with its ionic composition.

If in some way it were possible to prevent extensive expansion of the springs from their $K^+$ selective configuration, the general shape of an equilibrium diagram describing the system would be that shown in Figure 1, which is typical of any highly selective ion-exchange system. The higher the $K^+$ selectivity, the more the equilibrium curve crowds into the top left-hand corner of such a diagram. In the fully expanded $Na^+$ configuration, a separate equilibrium curve would crowd into the bottom right-hand corner of the diagram to an extent dependent on the $Na^+$ selectivity. There will be different equilibrium curves for solutions of different ionic strengths. The diagram shows that a large change in solution composition, from values corresponding to points such as $A$ and $B$ for example, will not change the $K^+$ content of the springs greatly; behind the region of point $A$ a large desorption of $K^+$ occurs for a small rise in the $Na^+$ content of the solution. If, as in the model, the assembly of springs is not prevented from expanding, some of the springs will begin to expand as their $Na^+$ content increases and their permeability will thereby change. A whole family of curves is therefore required to fully describe the properties of the spring.

The spring can exist in the contracted $K^+$ form or in the expanded $Na^+$ form according to the ionic strength and composition of the solution in contact with it, and the magnitude of the elastic restoring force. Increasing the latter, by increasing freedom for rotation of hydrocarbon chain segments, makes dilation more difficult and increases the stability of the $K^+$ form; the effect is analogous to increasing the crosslinking of an ion-exchange resin which also enhances the interaction energies of the exchanging ions (see Part I).

Fig. 1.—A hypothetical equilibrium diagram showing the composition of the springs, expressed as a mole fraction of the total anionic sites in unit area of the membrane, as a function of that of the solution in equilibrium with the springs, under conditions where expansion is inhibited.
In the presence of Ca$^{2+}$ and Mg$^{2+}$, as well as Na$^+$ and K$^+$, it is postulated that whereas the expanded springs are permeable to Ca$^{2+}$ and Mg$^{2+}$ the contracted springs are not so permeable. Because of its lower hydration energy, Ca$^{2+}$ is adsorbed in preference to Mg$^{2+}$ at the outer spring sites as an unhydrated, or partly hydrated, complex. Ca$^{2+}$, K$^+$, and spring elasticity therefore cooperate to enhance the stability of the contracted springs whilst Na$^+$ has a converse effect and promotes dilation.

The spring can open more rapidly than it can shut since contraction, but not expansion, involves some spatial rearrangement of the solvating components of the structure, and the displacement of water from K$^+$, in order to accommodate unhydrated K$^+$.

Unhydrated K$^+$, being bound to solvating groups attached to the immobile springs, has a lower mobility than does the hydrated Na$^+$ in the Na$^+$ configuration where the Na$^+$ is not so bound. The mobility of chelated Ca$^{2+}$ in the contracted configuration is also much lower than that of hydrated Ca$^{2+}$ ions in the expanded form. As the K$^+$ in each spring is accommodated within a single narrow channel across the membrane, it cannot traverse the membrane by an independent movement but only by successive displacement of the ions within the channel; as a consequence interchange of similar cations across the membrane is very small but becomes more extensive when the spring expands into the Na$^+$ configuration where cation mobility is much higher.

IV. Model of the Axon

The behaviour of a model of that part of a nerve fibre responsible for the action potential when immersed in seawater, and under voltage-clamp conditions, will now be considered.

Figure 2 shows a purely diagrammatic representation of the model. A number of springs are arranged across the membrane with seawater on the outside and a K$^+$-rich solution inside. Since the divalent ions Ca$^{2+}$ and Mg$^{2+}$ (referred to as M$^{2+}$) of the external seawater will be more strongly adsorbed than the univalent ions, as in ion-exchange adsorbents, it will be postulated that the two outer negative charges of most of the springs are associated with unhydrated or partially hydrated divalent ions which form an outer layer of adsorbed cations. Ca$^{2+}$ is thus adsorbed in preference to Mg$^{2+}$. Because of the high interaction energy between M$^{2+}$ and the spring sites, interaction of the latter with external Na$^+$ is very small [Fig. 2(a)].

Since the internal solution contains mainly K$^+$, only a little Na$^+$ and M$^{2+}$, and contact with external Na$^+$ is prevented by external M$^{2+}$, the remaining spring anions in the cavities containing the divalent ions will be occupied mainly by K$^+$, and the springs will be contracted. The membrane in the resting state is therefore mainly K$^+$ permeable to an outflowing current of K$^+$, but, because of the presence of adsorbed M$^{2+}$ and the low mobility of the associated K$^+$ and M$^{3+}$, the electrical resistance of the membrane is high and the membrane potential is not defined by the Nernst equation for the different K$^+$ activities on either side of the membrane. If K$^+$ ions are added to the external solution there will at first be little change in the membrane potential, but once the concentration of K$^+$ becomes sufficient to start replacing M$^{2+}$ by K$^+$ the membrane potential changes. When all the M$^{2+}$ has been replaced by K$^+$ the membrane behaves as a K$^+$ membrane and increasing amounts
of K\(^+\) change the potential in accordance with the Nernst equation. As a result of replacement of \(M^{2+}\) the membrane resistance is substantially reduced.

Because it is postulated that K\(^+\) within the membrane is confined to a narrow channel, and can move therefore only by a process of successive displacement as in the earlier model of von Kuhn and Ramel (1959), and because these ions provide the main conducting path through the membrane, the application of a depolarizing potential initiates an immediate movement in the channels of K\(^+\) which displaces outer \(M^{2+}\). Displacement of \(M^{2+}\) increases the electrostatic repulsive forces in the springs, which begin to expand and come in contact with Na\(^+\) [Fig. 2(b)]. The expansion, being rapid, occurs at first in preference to a slower, opposing process in which K\(^+\) in the springs tends to advance under the influence of the applied potential, and in so doing tends to maintain the springs in the contracted configuration as K\(^+\) displaces \(M^{2+}\).

![Diagram of membrane dynamics](image)

**Fig. 2.—** A diagrammatic representation of the state of the springs in a model nerve membrane under voltage-clamp conditions in the resting state (a), at the peak of the action potential (b), when Na\(^+\) current ceases (c), and under conditions when the outflow of K\(^+\) current has reached a steady-state value (d). The lower diagram illustrates the time relationship between the Na\(^+\) conductance (\(g_{Na}\)) and the K\(^+\) conductance (\(g_{K}\)) (after Hodgkin 1964).

The rate of movement of K\(^+\) through the spring is a function of the applied potential. The ease and speed of displacement of \(M^{2+}\) by the exciting current is a function also of the potential and of their concentration in the external solution. The replacement of some \(M^{2+}\) by Na\(^+\) in the external solution decreases the tendency for \(M^{2+}\) ions to be adsorbed, which favours their displacement by a cathodic current. Substituting some \(M^{2+}\) by Na\(^+\) therefore enhances the ability of the springs to expand and so favours excitation. If the outer solution is diluted, keeping the Na\(^+\) : \(M^{2+}\) ratio constant, the adsorption of \(M^{2+}\) relative to Na\(^+\) will be favoured owing to Donnan effects (Bauman and Eichhorn 1947), and the membrane properties will change accordingly. If the depolarizing potential is applied too slowly when the external solution is seawater, \(M^{2+}\) will not be displaced rapidly enough to enable the springs to expand, and the membrane will not excite.
As a result of the displacement of $M^{2+}$, and the expansion of the outer part of some of the membrane springs, Na$^+$ begins to move into the membrane in the direction of its concentration gradient. Metabolic energy has been expended earlier by the sodium pump in creating a Na$^+$ gradient to provide the driving force for such an inflow of Na$^+$ as soon as an increase in Na$^+$ permeability occurs. As the expanding springs come in contact with the Na$^+$, they expand fully into the Na$^+$ configuration. The Na$^+$ conductance, $g_{Na}$, of the membrane rises rapidly as more and more of the springs expand. The changing membrane conductance is particularly complex immediately after excitation. The displacement of $M^{2+}$ first raises the K$^+$ conductance of the spring which is only later reduced as it expands into the Na$^+$ configuration. The process cannot be described therefore by a simple replacement of a K$^+$ channel by a Na$^+$ channel.

The rapid inflow of Na$^+$ carries with it some $M^{2+}$, but, as the latter does not pass through the expanded form of the membrane as rapidly as does Na$^+$ because of its greater charge, $M^{2+}$ tends to accumulate near the outer edge of the springs, raises the local ionic strength, and so ultimately causes partial contraction [Fig. 2(c)]. Once the outer sites have contracted by reaction with $M^{2+}$ the inflowing Na$^+$ current through the spring ceases. Internal K$^+$ ions now displace Na$^+$ from the inner boundary layer and contract the inner side of the spring. When an outwardly flowing K$^+$ current now tends to reopen the contracted springs by displacement of $M^{2+}$, the situation is different from that at the beginning owing to the presence of the higher concentration of $M^{2+}$ and of K$^+$ in the vicinity. Hence a greater proportion of the springs than before will remain closed while K$^+$ advances and displaces the $M^{2+}$ to convert some springs fully into the contracted K$^+$ form. Thus the membrane Na$^+$ conductance passes through a maximum [Fig. 2(b)], but as $M^{2+}$ and K$^+$ accumulate, the outward K$^+$ current increases in magnitude as more of the springs close, and as K$^+$ displaces $M^{2+}$ from the springs [Fig. 2(c)]. Eventually K$^+$ displaces $M^{2+}$ from the boundary [Fig. 2(d)]. In such a state the springs have their maximum K$^+$ permeability and conductance so that the membrane potential is closer to the value of the Nernst K$^+$ potential, and the K$^+$ conductance is higher than at the beginning, when $M^{2+}$ was also adsorbed.

In this model, the process of "Na$^+$ inactivation" is that which results in the accumulation of $M^{2+}$ and K$^+$ and the conversion of the membrane completely into the K$^+$ form. The time for complete development of K$^+$ permeability will be longer than that for the removal of Na$^+$ permeability because of the additional time required to remove accumulated $M^{2+}$.

If, after a period of about 1 msec from the time of excitation, the membrane potential is restored rapidly to its original value, $M^{2+}$ will not have moved far away, and relatively few K$^+$ ions from inside will have entered the outer boundary layer. Hence the resting state condition is rapidly re-established, and the nerve can initiate a second action potential almost immediately. Under such circumstances, the rate at which the membrane returns to the resting state will increase with increasing concentration of $M^{2+}$ in the outer solution. If the reversal does not occur until 5 or 6 msec after the initial excitation, the outer boundary layer adjacent to the springs will contain mainly K$^+$, due to the outwardly flowing current of K$^+$, so that it will take some time for Na$^+$ and $M^{2+}$ to diffuse back. Hence there is a "refractory"
period before a further action potential can be initiated. If the concentration of $M^{2+}$ in the outer solution is increased, the rate of rise of the latter part of the K$^+$ current will decrease as it will take longer to displace $M^{2+}$. In such a system the rate of expansion and particularly contraction is more likely to depend on the ease of movement of the relevant polymer segments, and the concentration of $M^{2+}$ than on the concentrations of Na$^+$ and K$^+$.

Thus the behaviour of the model depends critically on the presence and concentration of $M^{2+}$ in the external solution. An action potential cannot be excited in the absence of $M^{2+}$. As its concentration increases beyond a lower minimum value, increasing concentration makes excitation more difficult, reduces the rate at which Na$^+$ enters the membrane, and reduces the magnitude of the Na$^+$ current, for a given excitation current. Increasing the concentration of $M^{2+}$ also delays the rate of development of the latter part of the outward K$^+$ current.

$M^{2+}$ also takes part in the rectification properties of the membrane, as the membrane will more readily pass an outward K$^+$ current than an inward current under steady-state conditions. $M^{2+}$ occurs only in the external solution to any appreciable extent, and the adsorption of $M^{2+}$, which hinders the current, will be promoted by an inflowing but not by an outflowing current. Some rectification can also be expected to arise as a result of the different K$^+$ concentrations on either side of the membrane.

Figure 1 shows that, provided the ionic strength of the solution in contact with the model springs is constant, their K$^+$ content in the resting, contracted state will not vary greatly over a wide range of composition (compare points A and B). The rate of diffusion of K$^+$ within the contracted springs will be much lower than that of those in a free solution. Hence increasing the inner K$^+$ concentration above a lower limiting level, under conditions of fixed ionic strength and with seawater on the outside, will not alter the rate of movement of K$^+$ through the model membrane when compared under conditions in which there is a constant potential difference across the membrane.

When the nerve model is excited under conditions which do not involve a voltage clamp, local currents flow from the site of depolarization and out through nearby polarized springs. The outflow displaces the divalent ions, initiates a new rise in Na$^+$ conductance as the process of Na$^+$ inactivation commences at the original site, and so propagates the action potential along the membrane. As the outflow of K$^+$ diminishes at the original site of excitation, owing to the advancement of the peak of the Na$^+$ conductance further along the membrane, Na$^+$ and $M^{2+}$ diffuse back into the boundary layer, $M^{2+}$ is readorsbed, and the membrane reverts to its original state.

The above mechanism does not take into account experimental observations (see Tasaki 1968) which show that nerve excitation is sensitive to internal anions according to their position in the lyotropic series. To account for such observations it is postulated, in an extension of a concept proposed by Tasaki, that the outer, second layer of lipid of the spring bilipid is attached to the supporting membrane by salt links involving the polar structures of the lipid and which are accessible to the inner but not to the outer solution. Salts in the former therefore tend to rupture the salt links by competitive adsorption. The resulting loss in lipid rigidity weakens the spring tension, which is strongest when the supporting structure has maximum
rigidity. Consequently isotonic dilution of the inner solution, or replacement of anions by weakly adsorbed anions, such as F\(^-\), increases spring tension. The resultant enhanced K\(^+\) interaction with the exchange sites reduces the rate of movement of K\(^+\) across the membrane and so prolongs Na\(^+\) inactivation and the duration of the action potential.

Action potentials can be initiated in nerves containing an inner solution of NaF and an outer solution of CaCl\(_2\) (Tasaki, Watanabe, and Lerman 1968). Such conditions yield maximum spring tension in the model. Na\(^+\) can be accommodated in the contracted springs as unhydrated ions, instead of K\(^+\), whereas hydrated Ca\(^{2+}\) ions carry inward current, in place of outer Na\(^+\) ions, when excitation replaces the outer chelated Ca\(^{2+}\) ions and expands the springs. Closure of the springs depends on accumulation of Ca\(^{2+}\) ions within the inner boundary layer and of Na\(^+\) ions, from outer currents involving adjacent contracted sites, within the outer boundary layers. The peak of such action potentials is therefore more delayed than those under normal conditions.

V. Experimental Evidence

The model accounts in a qualitative way for the action potential of a giant squid nerve (see Hodgkin 1964), and for bi-ionic potentials (see Tasaki 1968), and is consistent with many experimental observations.

The findings of Weidmann (1955), Frankenlæusser (1957), and Frankenlæusser and Hodgkin (1957) are consistent with the postulated role of Ca\(^{2+}\) in excitation. It is known also that the membrane is impermeable to Ca\(^{2+}\) in the K\(^+\) state, but that some Ca\(^{2+}\) passes through when it is in the Na\(^+\) state, although the Na\(^+\) permeability is much higher (Hodgkin and Keynes 1957). Increasing amounts of Ca\(^{2+}\) increase the rate at which Na\(^+\) permeability is reduced (Frankenhaeuser and Hodgkin 1957). Such observations support the postulated role of Ca\(^{2+}\) in reducing Na\(^+\) permeability and the expansion of a K\(^+\) pore into a larger Na\(^+\) pore with some Ca\(^{2+}\) permeability. Increasing external Ca\(^{2+}\), or proton, concentration increases the depolarization needed to elicit a given response of the nerve, and as the pH value decreases the maximum Na\(^+\) conductance decreases quickly and reversibly (Hille 1968); such effects are consistent with increased stability of the contracted state in the presence of Ca\(^{2+}\) or are the result of suppressing ionization of the spring anions through protonation.

The model is consistent too with the large decrease in membrane resistance during excitation (Cole and Curtis 1939), with enhanced interdiffusion of cations across the excited membrane (Tasaki 1963), and with recent perfusion studies where the internal ion concentrations and compositions have been altered (Baker, Hodgkin, and Meves 1964; Chandler and Meves 1965; Chandler, Hodgkin, and Meves 1965).

By analogy with rubber, increasing temperature increases spring tension and the stability of the K\(^+\) state. This accounts for the phenomenon of “heat block” and for excitation resulting from sudden cooling under threshold conditions (Spyropoulos 1965). “Cold” anaesthesia (see Shanes 1958) would result when the membrane is cooled below its glass transition temperature so as to freeze the bilipids into an immobile glass.
The initial heat release observed during the rising phase of an action potential (Abbot, Howarth, and Ritchie 1965; Howarth, Keynes, and Ritchie 1968) is accounted for in two ways. Part would be consistent with greater ordering of the spring bilipids during dilation (cf. rubber). The remainder could arise from replacement of Ca$^{2+}$ by Na$^{+}$ and K$^{+}$ since, although Ca$^{2+}$ has greater affinity than K$^{+}$ for a sulphonic-type exchange resin, heat is evolved when K$^{+}$ replaces Ca$^{2+}$ due to a large negative entropy change (Coleman 1952). The model is consistent also with the increased volume and greater orderliness of a nerve membrane, as observed with light scattering and birefringence measurements, at the peak of an action potential (Cohen, Keynes, and Hille 1968).

VI. Action of Drugs and Hormones

The thesis will now be developed that some drugs influence action potentials as a result of interactions with the spring bilipids so as to change their elasticity and resultant membrane permeability.

(a) Membrane Stabilization

Membrane stabilization ("anaesthesia") results when membrane stabilizers, heat, or cooling below a critical temperature range stabilize the resting K$^{+}$ state of the membrane (Frank and Sanders 1963; Inoue and Frank 1967).

The activities of general anaesthetics correlates with their solubility in benzene and some fluorohydrocarbons (Miller, Paton, and Smith 1965), and with their Van der Waal correction factor and molar refraction (see Featherstone and Muehlbachecher 1963; Vandam 1965). Pauling (1964) also observed a correlation with their ability to form gas hydrates, but commented that any other property involving intermolecular interaction energy would give equally satisfactory results. There is also a correlation with affinity of some monomolecular films for gaseous anaesthetics (Clements and Wilson 1962; see Felmeister, Amanat, and Weiner 1966), or for low concentrations of local anaesthetics and alcohols (Skou 1954, 1958; see Shanes 1963; Cuthbert 1967). The ability of some alcohols and steroids to stabilize red cell membranes against hypotonic haemolysis correlates also with their anaesthetic activity; at much higher concentrations lysis occurs. Most lysins exhibit anaesthetic properties in low concentrations (Seeman 1966). Some agents which cause lysis of lysosomes, such as alcohol, ether, and chloroform, increase diffusion rates through a bilipid membrane in order of their anaesthetic activity (Bangham, Standish, and Miller 1965; Bangham et al. 1967). Spin-labelling of membranes, which indicates membrane fluidity, also increases in the presence of local anaesthetics (Hubbell and McConnell 1968).

Such observations suggest that anaesthesia is related to the ability of anaesthetics to bind to lipid hydrocarbons so as to decrease lipid–lipid interactions and to increase chain mobility. Since this would also increase the rotational freedom of segments of the hydrocarbon chains it suggests that anaesthesia results from increasing membrane elasticity, which increases the stability of the K$^{+}$ state, and recalls the effect of small amounts of solvents in converting glass-like polymers into rubber (see Part I). At much higher concentrations of the anaesthetics, the hydrocarbon chains dissolve and the membrane ruptures (lysis).
Local anaesthetics are dibasic, one site being weaker than the other. There is a structural correlation between their potency and the bond order of the carbonyl portion of the ester group of the para-substituted benzoate ester, which suggests that the latter may participate in a charge-transfer complex (Feinstein and Pamire 1966). Such drugs form specific molecular complexes with diester phosphates, but not with monoester phosphates (Feinstein 1964; Feinstein and Pamire 1966), and there is a correlation between activity and their ability to displace Ca$^{2+}$ from phosphatidylserine (Blaustein 1967). Local anaesthetics displace Ca$^{2+}$ in nerves (Kuperman, Altura, and Chezar 1968). Their potency parallels their ability to inertact with, and to penetrate, a monolayer, as indicated by a rise in surface pressure (see above).

Studies of the ability of local anaesthetics to stabilize a nerve (Ritchie, Cohen, and Dripps 1965; Blaustein and Goldman 1966; Hille 1966) suggest the following mechanism. At low concentrations undissociated molecules penetrate the lipid phase where they function as swelling agents like the general anaesthetics. At higher concentrations, and being dibasic, they displace Ca$^{2+}$ from spring sites and, by crosslinking them, stabilize the K$^+$ state. Unlike Ca$^{2+}$, they are not readily displaced by an excitatory current because of additional interactions with the lipid. Figure 3 shows that the basic sites of tetracaine fit neatly along two anionic sites of the spring. In such a situation the channel is partly blocked so that the K$^+$ impedance is increased. High concentrations of Ca$^{2+}$ will tend to displace the anaesthetic. Consequently, removal of Ca$^{2+}$ ions by EDTA excites, but removal by reaction with local anaesthetics inhibits action potentials. Kuperman, Altura, and Chezar (1968) have recently drawn attention to this paradox. Since both lipid interactions with anaesthetics and spring crosslinking with local anaesthetics increase the stability of the K$^+$ state, the observations that both the unionized and ionized forms of local anaesthetics show activity (Ritchie and Ritchie 1969), and that an effective block can be obtained with a mixture of a cationic anaesthetic and a non-ionic anaesthetic, each being at half its minimal blocking concentration (Schauf and Agin 1969), are consistent with the hypothesis.

![Figure 3](image_url)
The hypothesis suggests an alternative mechanism for inducing anaesthesia in which the spring bilayer hydrocarbon chains are frozen into an immobile glass by reducing temperature to below the glass transition range (cold anaesthesia—see Shanes 1958), or by forming rigid molecular complexes with the contracted lipids and substances such as cholesterol and some other steroids which are known from surface-film and spin-labelling studies (Hubbell and McConnell 1968) to form such rigid complexes. Many steroids are good anaesthetics (see Kappas and Palmer 1963). Such reactions show much greater specificity than do those associated with general anaesthesia, since they depend on the ability of the agent to fit within the contracted membrane so as to bind with the lipid hydrocarbons and their polar groups. Steroids can induce anaesthesia in the model in two ways; some will form rigid complexes but others will inhibit by enhancing elasticity.

Tetrodotoxin is an exceptionally powerful anaesthetic, but does not displace Ca\(^{2+}\) like local anaesthetics (Kuperman, Altura, and Chezar 1968). It interacts with cholesterol and expands monolayers of nerve lipids containing cholesterol (Camejo and Villegas 1969), which suggests that it may enhance spring elasticity by neutralizing the condensing effect of cholesterol.

(b) **Membrane Labilizers**

Some substances, such as DDT and some alkaloids, stabilize the Na\(^{+}\) form of the membrane. It is postulated that in such situations the labilizers form specific molecular complexes with the lipid hydrocarbons and their polar groups, but that their stereochemistry is such that this is possible only with the dilated membrane. Unlike some steroids, which stabilize the contracted K\(^{+}\) state by forming complexes, these agents stabilize the expanded Na\(^{+}\) state.

Consistent with such an hypothesis is the fact that the ability of DDT, and some cyclopropane insecticides, to stabilize the Na\(^{+}\) state correlates with their size and shape (Mullins 1954, 1959; Holan 1969), which is such as to suggest that they function as molecular wedges within Na\(^{+}\) cavities, keeping the expanded spring sites open. Veratridine is a nerve excitant that forms strong molecular surface complexes with lipid monolayers (see Shanes 1963), which suggests that it may intercalate between the expanded bilipid layers of the spring and stabilize them. The related, but much flatter, derivative veratramine has the opposite effect of stabilizing the K\(^{+}\) state (see Shanes 1963) which suggests that it is sufficiently flat to complex with the contracted spring.

(c) **Drug Antagonism**

Since the essence of the hypothesis is the establishment of an equilibrium between dilatory and contractile processes in the membrane, it follows that effects arising from heating, cooling below the glass transition temperature, or additions of Ca\(^{2+}\), local anaesthetics, or general anaesthetics which increase the stability of the contracted state, should antagonize the effects of substances having a labilizing effect. Many examples of such drug antagonism are known and similar effects have been observed in lipid monolayers. For example DDT is antagonized by Ca\(^{2+}\) (Gordon and Welsh 1948), by heat (see Holan 1969), and by tetrodotoxin (Narahashi and Haas 1968). Ca\(^{2+}\) and Mg\(^{2+}\), tetrodotoxin, and local anaesthetics antagonize vera-
tridine (Shanes 1963; Ulbricht 1965; Baker 1968). The ability of veratridine to decrease the surface pressure of stearic acid monolayers can be opposed by addition of anaesthetics (see Shanes 1963).

(d) Rectification

Ca$^{2+}$ blocks an inward but not an outward membrane current and rectifies at the outer surface (Steinbach, Spiegelman, and Kawata 1944; Frankenhaeuser and Hodgkin 1957; Lecar et al. 1967). Some drug effects can be attributed to rectification. For example, tetraethylammonium, and particularly pentyliethylammonium, ions exhibit reversed rectification at the inner side of the membrane by tending to mechanically block outwardly moving K$^+$ currents; they allow inwardly moving Na$^+$ currents to pass. Subsequent addition of tetrodotoxin then blocks the Na$^+$ currents (Armstrong and Binstock 1965; Hille 1967; Armstrong 1968) by preventing dilation.

(e) Hyperpolarization

Some drugs, such as chlorpromazine (Brady 1964) and sodium pentabarbitol (Sato, Austin, and Yai 1967), depress the resting potential of nerve. This might be due to one of several possible mechanisms for hyperpolarization. Interactions which increase spring tension would increase ion interaction. Other possibilities are increasing the charge density of spring anions, or "ligands", by formation of charge-transfer complexes with the peptide spring component, which may be a semiconductor, or with the double bond of vinyl ether groups in plasmalogens whose oxygen atoms chelate K$^+$ (see Part I).

VII. Sensory Receptors

Sensory receptors may function as a result of changing spring elasticity through mechanical deformation, changing temperature, or adsorption of odours or tastes. The early receptor potential of light-sensitive organisms is associated with photoisomerization of the carotenoid side-chain of retinal from a highly sterically hindered, bent, and twisted 11-cis configuration to a stable, mobile, all-trans configuration (Wald 1968). The temperature dependence of the process suggests that the 11-cis isomer may be a glass which becomes a rubber when converted to the all-trans isomer. Davies (1965) has observed that a plot of the molecular cross-sectional areas of odours against their desorption energies from a lipid–water interface into water discloses discrete areas characteristic of odour types. Amoore, Johnston, and Rubin (1964) have found reason for classifying odours into seven shapes and for each they postulate a special receptor. A correlation has been noted between adsorption of odours on carotenoids and the electrical resistance of the latter (Rosenberg, Misra, and Switzer 1968), but the correlation could equally well reflect affinity for the carotene. A conformational change accompanies odour adsorption in yellow-brown scrapings from the olfactory epithelium (Ash 1968). As these almost certainly contain carotenoids it is possible that adsorption of odours on membrane carotenoids changes membrane elasticity and so influences action potentials. The many possible isomers of carotenoids would make them ideal receptors for such a process.
VIII. Discussion

The model is somewhat related to the earlier theories of Mullins (1954, 1959). He postulated a membrane in which the distribution of pore sizes strongly favours K⁺ transfer when there is no potential. Electrical asymmetry causes non-penetrating ions on the membrane capacitor to exert a mechanical force on both membrane surfaces, which deforms the pores so as to assume a distribution of sizes favouring the ions exerting the mechanical force. DDT was shown to have dimensions which would enable it to fit within a membrane pore which could accommodate hydrated Na⁺, and this was supposed to distort other pores into a Na⁺-selective state. The current model provides a simple explanation for the activity of DDT and so removes the paradox of DDT blocking Na⁺ pores.

The model is an extension of the ideas of a number of prior workers who have postulated also a role for Ca²⁺ in initiating an action potential (Hodgkin, Huxley, and Katz 1949; Karrenman and Landahl 1953; Huxley, cited in Frankenhaeuser and Hodgkin 1957; Tobias 1958; Mullins 1959; Goldman 1962; Tasaki and Shimamura 1962; Ehrenpreis 1964; Lettwin et al. 1964; Koketsu 1965; Watanabe, Tasaki, and Lerman 1967).

Frankenhaeuser and Hodgkin (1957) investigated an earlier concept of Hodgkin, Huxley, and Katz (1949), who had proposed that Ca²⁺ blocks the inner end of a Na⁺ permeable pore and is removed on excitation, but found by calculation that the rate of rise of the Na⁺ conductance was not rapid enough to account for the experimental observations. They discarded a suggestion of Huxley that Ca²⁺ blocks the outer surface of a Na⁺ pore because it could not explain the large effect of Ca²⁺ on the rate at which the Na⁺ conductance is shut off under an anode, or the increased Ca²⁺ entry associated with the conduction of impulses shown by Hodgkin and Keynes (1957), although it was admitted that the concept was otherwise in accordance with experiment. Although neither model could account satisfactorily for all the facts, Frankenhaeuser and Hodgkin (1957) concluded that “the general possibility that depolarization acts by removing Ca²⁺ from combination with a sodium carrier seems sufficiently plausible to keep in mind”. The concept of an expanding pore which contracts at higher Ca²⁺ concentrations removes these objections, and also the paradox that local anaesthetics inhibit by removing Ca²⁺ but Ca²⁺-chelating agents excite (Kuperman, Altura, and Chezar 1968).

The model resembles in many ways the “two-stable-states” theory of excitation by Tasaki which proposes that a conformational change of membrane macromolecules, initiated by the displacement of an outer layer of Ca²⁺ by the excitation current, is the primary event in excitation (Tasaki 1968). As in Tasaki’s theory, the suppressive effect in the external solution of large polyatomic cations containing hydrocarbon structures (e.g. tetraethylammonium) on the model is attributed to their ability to reduce the rate of conformational change in the membrane. In the model this is accounted for by their ability to inhibit expansion of the springs because of weak crosslinks induced by their attachment to the adjacent membrane structure by short-range interactions and by simultaneous electrovalent attachment to the exchange sites. Such a mechanism accounts also for the almost complete absence of inwardly flowing current. Small hydrophilic cations, such as hydrazine, cannot
bind to the hydrophobic membrane wall and can therefore substitute for Na\(^+\). The model proposes, as in Tasaki’s concept, that the key requirements for an action potential are the presence of divalent ions in the outer solution, and of monovalent ions in the inner solution, rather than Na\(^+\)-K\(^+\) selectivity, but the model accounts also for the observed Na\(^+\)-K\(^+\) selectivity of nerve and shows how it can facilitate the action potential. A further difference is that in the resting state the exchange sites are occupied by M\(^{2+}\) and K\(^+\), and not by M\(^{2+}\) and Na\(^+\) as in Tasaki’s concept, so that during excitation a change from an M\(^{2+}\)-K\(^+\) state to a Na\(^+\) state and then to a K\(^+\) state is postulated for the model whereas Tasaki proposes a direct change to a K\(^+\) state.

The recent studies of the antagonism between tetrodotoxin and DDT, or veratridine, have been interpreted as providing evidence for the presence of separate and independent Na\(^+\) and K\(^+\) channels rather than a single channel of varying selectivity (Hille 1966; Baker 1968; Narahashi and Haas 1968). This conclusion is based on an analysis of the experimental results in which, for example, a peak transient Na\(^+\) current is calculated by subtracting the current observed in the presence of DDT and tetrodotoxin from that observed in the presence of DDT alone, and assumes that the same K\(^+\) current flows in each case. However, the actual observation by Narahashi and Haas (1968) for example is that “it is clearly seen that the inward steady-state current (observed with DDT) has now been converted into an outward steady-state current” (observed with DDT plus tetrodotoxin). The model provides a direct explanation for such an observation.

A simple process by which a Na\(^+\) channel converts into a K\(^+\) channel is untenable because the rate of rise in K\(^+\) current would equal that of the decline of the Na\(^+\) current; experiment shows that the former is more prolonged (Baker 1968). In the model, closure of the Na\(^+\) configuration by accumulating Ca\(^{2+}\) gives rise to a Ca\(^{2+}\)-K\(^+\) pore when Na\(^+\) permeability has been turned off, and it takes an additional time for the K\(^+\) current to remove the Ca\(^{2+}\) which is necessary for maximum K\(^+\) permeability; hence K\(^+\) permeability rises more slowly than Na\(^+\) permeability decreases.

The model, in essence, is that of a permeability change induced by an exchange of two ions with different interaction energies with exchange sites in the membrane, in which the removal of another component induces a dilatory stimulus which excites by expanding the membrane, thereby changing its ion selectivity, and is followed by a contractile stimulus which contracts the membrane and restores the original permeability. It therefore resembles in principle the model poly(acrylic acid) membrane of Walters, Kuhn, and Kuhn (1961) where an action potential was initiated by a dilatory stimulus of sodium hydroxide followed by a contractile stimulus of hydrochloric acid; the changes in membrane potential observed reflected the changes in the H\(^+\) : Na\(^+\) composition of the membrane.

Because of the large amount of evidence suggesting a role for Ca\(^{2+}\) in nerve excitation, such a role has been adopted in the model, but a Wien effect, which changes membrane pH, could be involved as well as has been proposed by Bass and Moore (1968). The marked pH sensitivity resulting from strong ion association with K\(^+\) in the contracted springs (see Part I) would favour such a mechanism. If some of the exchange sites are protonated (cf. the sodium pump model in Part III, Weiss 1969c), the addition of alkali would enhance their ionization, thereby expanding
the springs and exciting the membrane. An increase in the internal pH value of about 0.2 is known to cause excitation (see Bass and Moore 1968).

The concept of adsorption interactions with spring bilipids influencing a dilatory–contractile equilibrium by changing membrane elasticity provides a simple and consistent explanation for the influence of membrane stabilizers and labilizers on action potentials and for the behaviour of some sensory receptors. The model provides a specific example of the general principles outlined in Part I.

There is no evidence that Heald's phospholipoprotein (1961a, 1961b, 1962; see Part I) is involved in the action potential, although there is evidence, to be discussed in a later paper, that phosphatidylserine is a component of the sodium pump for which a closely similar spring will be postulated. According to Moore, Narahashi, and Shaw (1967) there are only about 13 Na+ channels per square micrometre in lobster nerve membrane, which emphasizes the formidable problem of chemically identifying them. It would be useful to study Heald's preparation, but with lipid attached, to see if its cation selectivity changes with changing conformation, as with polylysine. It might also be incorporated within synthetic bilipid membranes for further study.

It is concluded that the model provides a reasonable qualitative explanation at the molecular level of the action potential in squid by postulating an equilibrium between dilatory and contractile processes. It remains to be seen if the empirical parameters in some of the existing quantitative descriptions of the action potential can be modified to conform with it.

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X. References

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ENERGY TRANSDUCTION IN BIOLOGICAL MEMBRANES. II
