An Analysis of Pore Size in Excitable Membranes

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INTRODUCTION

It is possible to develop a method of analyzing membrane pore sizes based on quite different principles from those so elegantly described by Dr. Solomon in this Symposium. This depends upon an assumption that ions in aqueous solution will not penetrate through a membrane with pores of less than 10 A in radius unless the size of the ion and the size of the pore are almost equal (1). Such a situation makes it possible to equate the permeability of the membrane for an ion to the number of pores in the membrane that just fit an ion of a particular size. The conclusion from such an analysis is that the mean pore radius in excitable membranes is about 4.0 A; this value is in agreement with values deduced for the red cell membrane from quite different considerations (2). The advantage of considering excitable membranes and their pore size distributions is that the marked changes in ion selectivity and conductance exhibited by such structures offer a severe test of any hypothesis offered as an explanation for the operation of such membranes.

It appears that the cycle of permeability change exhibited by the squid axon under voltage clamp conditions accounts quantitatively for the action potential that is observed (3). It also seems a valid generalization that most electrically excitable systems rely on a similar cycle of permeability change whereby the membrane becomes selectively permeable first to Na^+ and later to K^+. There are, however, exceptions to this mechanism: crayfish muscle is normally not electrically excitable, but is able to give an action potential under conditions in which most of the extracellular cation is Ba^{++}. It seems likely that the depolarization observed is a consequence of an inward Ba^{++} current produced in response to a depolarization of the membrane (4). Barium is also apparently able to serve as a current carrying ion in vertebrate B and C fibers (5). It is also of interest to note that Ba^{++} and K^+ have the same crystal radius. A second system is the plant cell *Chara*, where the membrane is most permeable to K^+ but the response to depolarization is a great increase in chloride permeability of the membrane (6), and an increase in potassium permeability. The sodium permeability is unaffected by changes in membrane potential.

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Even when a particular excitable tissue depends primarily upon a cyclic Na^+–K^+ permeability change there are significant differences. For the frog muscle fiber membrane, it is clear that P_{Na} and P_K both increase greatly during a spike (7) but the Na^+ influx per impulse is about 50 per cent greater than the K^+ efflux, suggesting the necessity for an increase in P_{Cl} during the falling phase of the spike. Measurements of the spike shape in solutions not containing Cl^- also suggest that such ions are necessary for prompt membrane repolarization (8). Finally, although an increase of P_K in muscle is necessary to explain the flux data, it is also clear that such an increase is not sustained, as it is in squid axon, when the membrane potential is held around zero (9, 10) because the potassium permeability (from measurements of K^+ influx) is about the same at all values of the membrane potential less than -90 mv. While the details of the permeability changes with excitation remain to be worked out, it appears that in muscle, an increase in P_{Na} is followed by an increase in both P_K and P_{Cl}, and that all these permeability changes are transient.

Studies of myelinated nerve at the node of Ranvier show that while under voltage clamp the ion currents are similar to those in squid (11), there are variations in current patterns induced by experimental treatment; one of the more impressive of these is the effect of low concentrations of Ni^{++}. This treatment prolongs enormously the duration of the action potential with no effect on the amplitude but Ni^{++} have no effect on the spike of the squid axon (12).

There is also evidence that while the frog muscle fiber is more permeable to Cl^- than to other halide anions (13) (and indeed it is more permeable to Cl^- than it is to K^+, (14)), heart muscle fibers are more permeable to I^- than to Cl^- (15), and an inference from the pharmacological effects of Br^- in animals is that CNS cells are more permeable to Br^- than to either Cl^- or I^-. The foregoing serves to emphasize that the specificity of the membrane with respect to the species of ion that it will pass is capable of wide variation. This discrimination takes place, furthermore, between ions that are chemically very similar.

Any explanation of ionic phenomena in excitable tissue that aims at being at all comprehensive must allow for the fact that the resting membrane can be permeable principally to an anion, and that excitation transforms the membrane into one specifically permeable to Na^+, later to K^+, and possibly also to Cl^-, as well as the reverse situation where the membrane is principally permeable to a cation, and is transformed into an anion-permeable structure during excitation. A proper explanation must also deal with the means whereby ionic selectivity is maintained, and with the means whereby the conductance (or permeability) changes of the membrane are brought about. It is at present not possible to give such a comprehensive explanation and the purpose of this paper is to indicate some of the reasons for our ignorance regarding the ionic phenomena involved in bioelectric systems.

DISCUSSION

The Permeability Coefficient A constant that defines the relationship between flux of an ion species and the driving force bringing about the flux is customarily called the permeability coefficient. There is the difficulty with permeability coefficients for ions that they are not obtainable without assumptions regarding the distribution of the electric field within the membrane. Constant field assumptions (16) are almost always used but the predictions of such equations are almost never in agreement with experiment. In squid axon the K^+ fluxes change very much more with membrane potential than would be predicted by the equations and it is necessary for the permeability of the membrane to change with potential. In muscle, the anomalous rectification (17) makes it necessary for P_K to vary depending upon the direction of the net flux of the ion (14). Such findings as these serve to emphasize the precarious theoretical basis upon which ion permeability measurements are founded; it is perhaps safer at present to regard ion permeability coefficients as purely empirical indices of the ease of ion movement under carefully defined conditions. The conventional permeability constant is the product of a partition coefficient and a mobility term. For a homogeneous membrane it is clear that the partition coefficient sets the boundary conditions of concentration at both sides of the membrane while the mobility, multiplied by RT/F, has the dimensions of a diffusion constant for the ion within the membrane. In a pore membrane, terms such as partition coefficient and ion mobility are less easily defined, and even less easily related to diffusion theory. As there are several phenomena exhibited by physiological membranes that are most easily explained if ions are required to move through pores and "in file," it may be that the mechanics of the translation of ions through pores are responsible for some of the peculiarities of physiological membranes.

For the purposes of this discussion, the partition coefficient of ions to the membrane will be taken as the fraction of pores that contain a particular ion per unit concentration outside as the outside concentration approaches zero. The reason for this specification is that it is found experimentally that the permeability coefficient of an ion, as deduced from constant field considerations, varies with concentration outside (10). The mobility is here considered to be the ease with which an ion can move along a right cylindrical pore. It will appear later in the discussion that the separation of an ion from dilute aqueous solution, where it has an unlimited ability to be hydrated, to a membrane pore where only a restricted number of water molecules can accompany the ion, involves a passage over a potential energy barrier greater than that involved in aqueous diffusion. This effect occurs at both membrane surfaces and is conveniently included in the mobility term for the ion. The mobility may be a

very discontinuous function of the distance that the ion has moved through the membrane.

Ion Size and Penetration Rate Several experimental investigations aimed at discovering a relationship between alkali cation size and rate of penetration of the membrane of frog muscle fibers (10, 18) are in agreement in finding that the rate, at constant membrane potential, is lowest for Na^+, increases greatly for K^+, and then diminishes for Rb^+, and even more so for Cs^+. Such findings are difficult to account for on the basis that it is limiting equivalent conductance of the ion in aqueous solution that is a measure of ion size,[1] because K^+, Rb^+, and Cs^+ all have very close to the same limiting conductance and therefore must have very much the same "hydrated ion radius."

An alternate way of explaining the experimental facts (18) has been to suppose that: (*a*) the membrane is composed of pores with some dispersion in pore size, (*b*) an ion that is to penetrate the membrane must take along with itself some integral number of complete layers of water of hydration, and (*c*) an ion cannot remain in a membrane pore unless the hydrated ion fits the pore closely. This last specification requires some comment because while it is easy to see that an ion cannot penetrate into a pore that is smaller than the ion, it is not common experience that an ion is prevented from penetrating through a pore that is just a little too large. Such a consideration arises because the ion, with a single layer of hydration, has by no means saturated its demand for hydration, and in fact the first shell of water molecules around an ion is bound with a heat corresponding to roughly half of the total heat of hydration. As the heat of hydration of Na^+ is roughly 100 kcal./mole, this means that such an ion with a single layer of hydration still has a residual demand for hydration corresponding to 50 kcal. It cannot, therefore, be removed from solution without reference to this further hydration demand. If a pore in the membrane does not fit the ion closely, there exists a space around the ion where the dielectric constant is 1 and hence a region where the electric field of the ion is unshielded. The situation is thus similar to that encountered in attempting to dissolve ions in solvents of low dielectric constant in that the number of ions that can exist in such a solvent is extraordinarily minute and is limited by the fact that the electric fields of the ions are unshielded by the solvent and hence the predominant action is an attraction between positive and negative charges to produce ion pairs. The result is that the partition coefficient for ions to membrane pores where there is not a close fit is extremely small compared with the partition coefficient to pores where there is a close fit.

Membrane Pore Area Squid axons under especially good experimental conditions show a membrane resistance of the order of 2 ohm cm.^{2} during depolarization (19); if the membrane is 100 A thick, this is a specific resistivity

[1] For a detailed review of the meanings of this term see reference 26.

of 2×10^6 ohm cm., and is to be compared with the specific resistivity of sea water (20 ohm cm.), from which it differs by a factor of 10^5. To account for this difference in specific resistivity, it might be supposed that 10^{-5} of the area of the membrane is aqueous, and permits the passage of ions. If this area were concentrated in a single pore per square centimeter, it would have a radius of 1.7×10^5 A. The electrical consequence of such an arrangement would be easily detectable experimentally and it would be difficult to imagine that the membrane could retain any sort of ion selectivity. It is necessary, therefore, to regard the factor of 10^{-5} as reflecting the contribution to the ion conductance that is made by a number of pores distributed more or less uniformly over the surface of the membrane.[2] We have then to choose between a small number of large pores and a large number of small pores. Such a choice must involve consideration of the following: if we take pore radii as of the order of 50 to 100 A, the membrane might be expected to resemble collodion membranes (20) which have a selectivity for ions that, as will be shown later, does not follow the order observed in natural membranes. Even greater difficulties arise in attempting to account for conductance changes in such large-pore membranes. If the pores are made very numerous and of the order of 1 to 2 A in radius, calculation shows that all of the pores are too small to admit a hydrated ion, and such a membrane should be expected to have an infinite resistance. Clearly the choice of an ion radius must lie between these limits, and it is the purpose of the following discussion to examine this problem.

Membrane Pore Size Distribution An assumption of equal mobility for all monovalent cations in the membrane makes it possible to relate rates of penetration of such ions with the number of pores in the membrane of a given size.[3] A more elaborate mechanism is one where the mobility of ions in the membrane depends upon the extent to which membrane pores are filled with ions (1); a high mobility results from establishing pores along which chains of sites are completely occupied by ions of a given sort. Properly arranged, such a mechanism is capable of rectification and of large conductance changes but present experimental information is insufficient for the separation of permeability measurements into partition coefficient or mobility parts. The treatment below assumes that if the external concentration of a particular ion is made sufficiently low, the membrane is not greatly disturbed by the presence of such

[2] It should be noted further that diffusion through pores of the same order of size as that of hydrated ions cannot be expected to resemble aqueous diffusion in any quantitative respect. For this reason it is not possible to relate membrane resistivity to any absolute value for pore density unless the pore radius is so large that diffusion is as free as aqueous diffusion.

[3] It might appear that this is not a valid assumption because the hydration energy of the ions is included in the mobility and this differs considerably from one ion to another. Such a difference in hydration energy in solution is likely to be compensated because of a stronger solvation energy in the membrane.

an ion outside and that it passes the ion largely on the basis of the number of channels of appropriate size that are available.

The influx of a monovalent cation at constant membrane potential and per unit external concentration is proportional to its permeability. With the assumption of equal mobility for all alkali cations, the permeability coefficient is proportional to the partition coefficient. With the assumption that an ion must fit a pore closely if it is to be partitioned to it, we need only to plot permeability of the membrane for various ions against the size of the ion in order to ob-

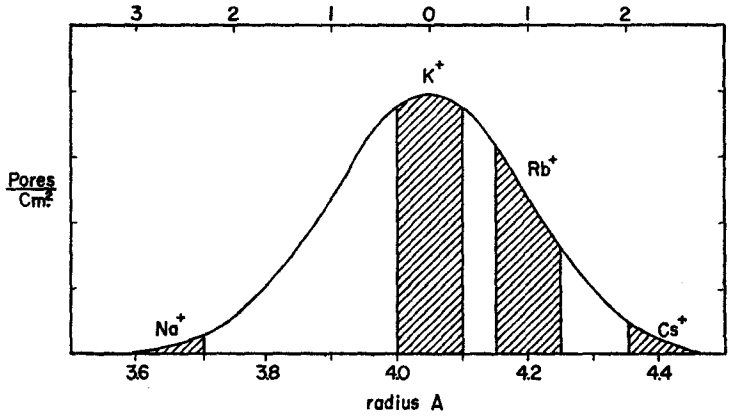

FIGURE 1. The shaded areas represent the permeability of the membrane of frog muscle fibers for the ions shown, as well as the number of pores in a distribution that fit an ion to within ±0.05 A. The smooth curve is the distribution of pore sizes in the membrane and is drawn with a standard deviation equal to 0.15 A, and with a mode at 4.05 A. The ordinate is both ion permeability and the number of pores per unit area of membrane. Abscissa is ion radius (crystal radius + 2.72 A) or pore radius. At the top of the diagram the abscissa is standard deviation. (Reprinted from *J. Gen. Physiol.*, 1959, 42, 817.)

tain a curve that will also represent the distribution of membrane pore sizes. Fig. 1 shows the results obtained from the alkali cations penetrating into frog muscle. This is a Gaussian curve with a mode at the size of K^+, and has been constructed on the basis that it is the ion with one complete shell of water molecules that is the penetrating entity. Other bases for the estimate of the size of the penetrating particle would, however, give comparable results.[4]

The fact that the permeability of the muscle cell membrane for various ions can be represented as a Gaussian distribution of pore sizes in no way proves the hypothesis advanced; it is perhaps better to point out that the hypothesis is physically reasonable and that it has better predictive value than the use of

[4] It should be noted that the order for the permeability of K^+, Rb^+, and Cs^+ is different from that usually found for oxidized collodion membranes or for ion exchange systems. This is most easily understood by supposing that the total dehydration of the ion by binding to an exchange site is not involved in the penetration of physiological membranes. Anion exchange systems also show a selectivity that is opposite to that found in organisms.

"hydrated ion radii" derived from conductance data. Further experimental support for the mechanism of ion penetration that has been proposed can only come from a demonstration that the penetration of all ions can be accounted for by the scheme. Multivalent ions of all sorts must be excluded for reasons that will be developed below, and anions with a different orientation of the water molecules in the hydration shell also must be excluded. The ions that could be used as test substances are shown in Fig. 2 where the crystal radii of a variety of ions are represented as ordinate and the ions are grouped somewhat

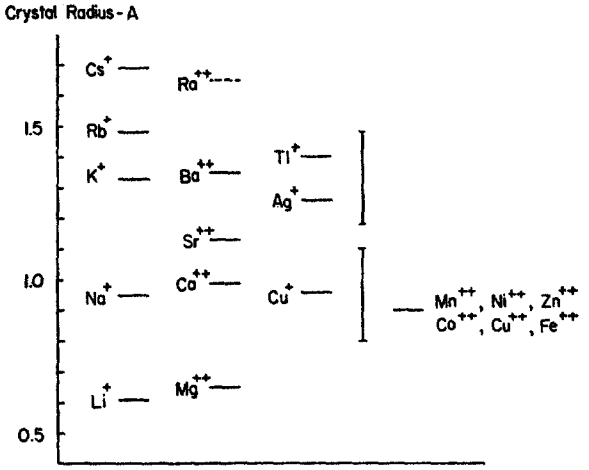

FIGURE 2. The crystal radius of various ions is shown as ordinate. Values for the alkali cations, and the alkaline earth cations are from Pauling (23) except for the value for Ra^{++} which is extrapolated. Values for other ions are from Goldschmidt (25) or from Hush and Price (24). The upper vertical bar represents one standard deviation of an error curve with a mode at the size of K^+, and lower vertical bar, is for a similar curve based on Na^+.

according to similarity of chemical properties. The vertical bars represent one standard deviation for an error curve with a mode at either the size of Na^+ or K^+, as these are the sorts of pore size distributions that are of physiological interest. Aside from the alkali cations, the only monovalent cations with chemical stability in this valence state are Ag^+ and Tl^+. Both ions are known to be toxic, but tracers make it possible to work at very low concentrations so that the relevant consideration in choosing between them was to select the ion forming a minimum of complexes, or insoluble compounds. This was necessary because any chemical reactivity either with the membrane or with components of the cytoplasm makes the analysis of the fluxes uncertain. On such a basis Tl^+, with generally soluble compounds, was much to be preferred. Thallous ion has a crystal radius of 1.44 A and is therefore between that of K^+ (1.33 A) and Rb^+ (1.48 A). Measurements showed (21) that per unit of concentra-

tion both the influx and efflux of Tl^+ in muscle were very similar to measured values for K^+ fluxes, that Tl^+ became distributed between fiber water of muscle and the external solution according to a Donnan ratio that is similar to that for K^+, and that Tl^+ depolarized the membrane about 58 mv. for a tenfold increase in external concentration. It would appear, therefore, that the crystal radius of a monovalent cation does, in some manner, determine its penetrability. Put another way, the membrane cannot apparently discriminate between Tl^+ and K^+.

The Penetration of Divalent Cations We know far too little about the structure of the hydration shells around the alkali cations, hence to press such an analysis to the much more complicated situation involved with the divalent alkaline earth cations is hardly profitable. What does seem clear is that divalent cations such as Ca^{++} carry insignificant currents across the membrane of the squid axon because the permeability of such ions, even under conditions of repetitive stimulation, is only about $\frac{1}{100}$th that for Na^+. It seems, therefore, that Ca^{++} while not directly involved in the charging or discharging of the membrane capacitor, has a regulatory action on the membrane permeability (22) and indeed its physiological action on axons can be accounted for on this basis.

The crystal radius of Ca^{++} is very similar to that of Na^+ and, on the basis of the hypothesis previously discussed, both of these ions should be expected to use pores of the same size for their transit across the membrane. Why then is the flux of Ca^{++} at constant membrane potential so very much smaller than that of Na^+ on the basis of equal concentration outside? The heat of hydration of Ca^{++} is about four times that of Na^+, and it is plausible to attribute the much lower permeability of Ca^{++} to the much less frequent escape of singly hydrated Ca^{++} from the water structure.[5] It is not unlikely that Ca^{++} escape from hydration by an even more complicated stepwise process of shedding hydration than for singly charged ions. A possible intermediate in this process is the interaction of one charge of Ca^{++} with the dielectric of the membrane, while the other charge is still involved with water molecules in the outside solution. This intermediate would have zero mobility with the result that Ca^{++} would remain at the entrances to membrane pores. Such a situation leads to

[5] It can be calculated that for Na^+, with a crystal radius of 0.95 A, a singly hydrated radius of 3.67 A with 6 water molecules in this shell, has about 30 water molecules in its second shell of 6.39 A. These are bound with an energy of 25 kcal./mole or 0.83 kcal./mole/water molecule. If the distribution of binding energy of Ca^{++} for water molecules in the second shell is the same as for Na^+, then the heat of hydration of Ca^{++} (400 kcal./mole) multiplied by 25/95, the fraction of Na^+ hydration energy by which the second water shell is bound, gives 105 kcal. or 3.5 kcal./mole/water molecule. The difference in energy required for a singly hydrated Na^+ or Ca^{++} to escape from one water molecule in the second shell is 2.73 kcal. and if the relative rates of escape of such ions depended upon such a step their ratio would be given by $e^{-2.73/RT}$ which at 300°K. has the value 0.01. This is close to the experimentally measured ratio for rates of penetration of Na^+ and Ca^{++}.

(*a*) a greatly decreased mobility of Ca^{++} over Na^{+}, and (*b*) an enhanced partition coefficient for Ca^{++} over that for Na^{+}. Low, or even zero mobility for an ion can result not only from a high hydration energy (to be expected for multiply charged ions) but also because the ion may be attached to an organic moiety that is itself too large to penetrate through a membrane pore. Such ions may be partitioned to the membrane, and so influence pore size distribution, even though their permeability is zero.

The Kinetics of Ion Movement into Membranes An ion, as it passes from aqueous solution into a membrane pore, becomes subject to a medium with a different dielectric constant and to one in which there may be a strong electric

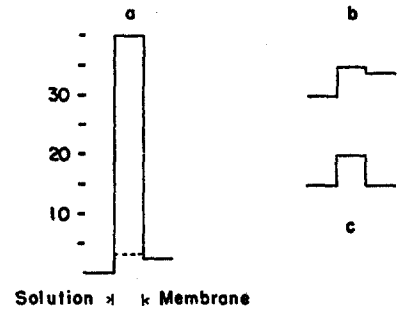

FIGURE 3. The ordinate is hydration energy in kcal./mole and the abscissa is the position of the ion from aqueous solution on the left through a transition region to membrane pore on the right. In *a* the dashed line indicates the energy an ion must have if it can enter a membrane pore by a stepwise removal of hydration in its second shell. The solid line shows the energy an ion must have if it is to leave aqueous solution for a medium where the dielectric constant is 1. Figs. *b* and *c* show the energy diagrams for the uncharged, and for the polarized membrane respectively.

field. Other complications that will not be considered here are the possibility of a large phase boundary potential, and the presence of other ions in the immediate vicinity. Fig. 3 has been drawn to show the situation at the entrance to a pore. The ordinate is hydration energy in kcal./mole and the abscissa is the position of the ion from solution outside the membrane to the interior of a membrane pore. In *a* the potential energy barrier is 40 kcal., corresponding to the work necessary to remove a mole of singly hydrated Na^{+} from solution. The membrane pore is supposed to have a dielectric constant somewhat less than water, hence the energy level in the pore is made, arbitrarily, 2 kcal. It is also supposed that the ion can detach itself from water molecules in the second hydration shell in a stepwise manner and that the dashed line represents the maximum energy that an ion must have to escape to the pore. The potential energy diagram for the membrane at zero potential is therefore that shown in *b*. If an electric field of appropriate sign is present within the membrane, this

will act to retard the movement of a cation in the direction membrane to solution but will have no effect on an ion moving in the opposite direction and the diagram for a charged membrane is that shown in *c*. The rate of ion movement in either direction ought to be connected with these considerations. Barriers of this sort may be negligible compared with others encountered by a penetrating ion, but these may be of decisive importance for a non-penetrating ion.

Pore Size Distribution in the Active Membrane The foregoing considerations suggest that any change in the species of ion that the membrane will pass (*e.g.* from K^+ to Na^+) indicates that the mean pore size of the membrane has

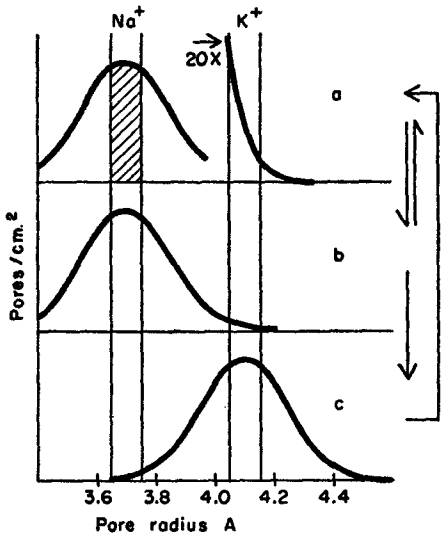

FIGURE 4. This is intended to show the changes in both conductance and pore size in the membrane of the squid axon at rest and during excitation. In *a* the membrane is at rest, and the hatched area under Na^+ size indicates occluded pores. Because the K-sized pores open would hardly show on the diagram, the ordinate has been magnified 20-fold for pore radii greater than 4.0 A. The resting conductance is almost entirely accounted for by these pores. The arrows between *a* and *b* indicate that the step from polarized membrane to depolarized membrane with no change in mean pore radius is entirely reversible. In *b* the membrane has just been depolarized with the result that Na pores are open and K pores are unchanged. The mean pore radius begins to shift toward K size and this movement is complete in *c* which gives the pore size distribution in the steady, depolarized state. To revert to the resting state (*a*) it is necessary to occlude pores with Na-sized plugs (step *c* to *a*).

changed. According to this view, the specificity of the membrane with respect to the sorts of ions that it will pass is solely a matter of pore size. Similarly, any change in the permeability of the membrane is to be related to the number of pores that are available for the passage of an ion. The experiments with Tl^+,

discussed previously, were extended to stimulated muscle and the results clearly showed that the efflux of Tl^+ from muscle was greatly increased during stimulation, and was slightly greater than the measured efflux for K^+ per unit concentration, per impulse, and per square centimeter. This result suggests that the active membrane uses the same means for discriminating between ions as does the resting membrane and that in neither case can Tl^+ be differentiated from K^+.

In any real membrane it must be supposed that there is a fixed number of pores per unit area and that these can vary within limits as to their size and as to whether they are occluded and therefore unavailable for the passage of ions. The cycle of conductance and ion selectivity change that takes place in the squid axon can therefore be represented as shown in Fig. 4. In *a*, the resting pore size distribution is shown. This diagram includes the following experimental observations: the conductance of the membrane (and therefore the number of pores open) is only about $\frac{1}{100}$th that of the maximal membrane conductance, the selectivity of the membrane strongly favors K^+, and the majority of the pores, although held at the size of Na^+, are plugged and therefore unavailable as pathways for Na^+ movement. This last assumption is dictated by the speed with which the Na^+ conductance, g_{Na}, can be switched on or off. It seems unlikely that, from what one knows of membrane time constants, pores could be altered in size quickly enough to bring about the increases in g_{Na} that are observed. In Fig. 4 *c*, the membrane is at zero potential and has reached a steady-state with respect to conductance; the curve reflects a selectivity that strongly favors K^+, and a conductance that represents a maximal removal of plugs from the pores. It is useful, in considering the properties of excitable membranes to start with the depolarized membrane and assume this to reflect the intrinsic conductance (or pore density) and ion selectivity (or mean pore radius) of the system. On this basis, the effects of polarization are twofold: the conductance may be decreased if increases in membrane potential lead to the occlusion of the pores with charged particles, and the mean pore radius may be changed if the occluding particles differ in size from K^+. This latter effect results in the membrane becoming mechanically strained so that it will revert to its zero potential pore size distribution when the occluding particles are removed from the pores. In Fig. 4 *b*, the membrane has been suddenly depolarized; this results in a rapid loss of the particles occluding the membrane pores, and in a slow reversion of the mean pore size of the membrane to that shown in *c*. The result of these two processes, acting with vastly different time constants, is a rapid rise and a slower fall in the sodium conductance of the membrane. Note that the step *a* to *b* is reversible in agreement with the notion that the occluding particles can be removed or replaced very quickly by changes in the electric field. Step *b* to *c* is not reversible in this sense because the occluding particles can only enter pores of Na^+-size and if there are none of these avail-

able (as in *c*), it is necessary to alter the membrane pore size (step *c* to *a*). This is a slow process and one which should be comparable in speed to that of step *b* to *c*.

An understanding of the changes in both ion selectivity and conductance in the squid axon reduces to one of discovering the identity of the particles that occlude membrane pores under conditions of membrane polarization. Some progress in this direction can be had by considering several hypothetical situations. Suppose, for example, that a membrane has a pore size distribution that favors the passage of Na^+, that this membrane separates two solutions Na^+, $Cl^-//K^+$, X^-, and that Na^+ and X^- are of the same size while K^+ and Cl^- have different sizes and are therefore non-penetrating. Suppose that the equilibrium potentials for Na^+ and X^-, are both $+40$ mv. and that the membrane potential is held at -70 mv. there is, of course, an equal driving force on both ions at all values of the membrane potential. If the membrane is 100 A thick, and the pores are of a size such that ions must move "in file," it would appear that a large number of the pores would become rapidly occluded because an anion and a cation would start out at opposite ends of the same pore and meet somewhere in the middle. Under this circumstance, the electric field of the membrane and the coulomb attraction of the ions act in concert. This effect should become more and more marked as the membrane potential becomes more negative, and should lead to a decreased conductance of the membrane. When the membrane potential is more positive than the equilibrium potential of the ions, there should be relatively little effect on the conductance because of the low concentrations of Na^+ and X^- on the opposite sides of the membrane.

A variation of the foregoing is to suppose that X^- is an ion with either a very low, or zero mobility in the membrane, and that there exists on the other side of the membrane a cation Y^+ which is the same size as Na^+ but which also has a very low mobility in the membrane. Providing that Y^+ is a strong competitor with Na^+ for pores, the principal ions in the polarized membrane will be Y^+ at the outside, and X^- at the inside. From a knowledge of the ions that exist in squid axon, it is tempting to suggest that Y^+ is Ca^{++} and that X^- is isethionate$^-$.

SUMMARY

The cell membrane has been treated as a small (less than 10 A radius) pore structure, and ion penetration has been postulated to require a good fit between the pore wall and the ion that is to penetrate. The pore radius of an excitable membrane at rest can therefore be analyzed by measuring its permeability to ions of graded size. The alkali cations appear suitable for such measurements, and an extension of such measurements to Tl^+ confirms the pore

size obtained with alkali cations. An advantage of the pore hypothesis is that it can be adapted to membranes with different ion permeability characteristics by merely altering the mean pore radius. Divalent cations have such large heats of hydration that it must be expected that the number of such ions able to escape from water into the membrane will be very much less than the corresponding number for singly charged ions. The selective permeability of the active membrane involves a change in mean pore radius of the membrane, while ion conductance changes arise because of competition of oppositely charged ions for pores of the same size. Conductance falls when large, appropriately directed, driving forces are exerted on such ions, and rises when the net driving force is zero. Further progress in elucidating the mechanisms operative in excitable systems probably will depend as much on obtaining a better understanding of the physical chemistry of ionic movement as upon further physiological information.

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