# THE PENETRATION OF SOME CATIONS INTO MUSCLE*

By L. J. MULLINS

*(From the Biophysical Laboratory, Purdue University, Lafayette, Indiana)*

(Received for publication, October 29, 1958)

ABSTRACT

The influx of $Na^+$, $K^+$, $Rb^+$, and $Cs^+$ into frog sartorius muscle has been followed. The results show that a maximum rate is found for $K^+$, while $Na^+$ and $Cs^+$ penetrate much more slowly. Similar measurements with $Ca^{++}$, $Ba^{++}$, and $Ra^{++}$ show that $Ba^{++}$ penetrates at a rate somewhat greater than that of either $Ca^{++}$ or $Ra^{++}$. All these divalent cations, however, penetrate at rates much slower than do the alkali cations. The results obtained are discussed with reference to a model that has been developed to explain the different penetration rates for the alkali cations.

## INTRODUCTION

It has been known for some time that, for frog muscle, $Rb^+$ and especially $Cs^+$ penetrate at rates substantially less than that for $K^+$ (1), although for invertebrate tissues potential and conductance measurements suggest that the rate for $Rb^+$ is greater than that for either $K^+$ or $Cs^+$ (2). It is also clear that frog muscle shows less depolarization per unit of external concentration with $Rb^+$ in Ringer and much less depolarization with $Cs^+$ in Ringer.[1] These experimental findings make it difficult to subscribe to the usual explanation for the low rate of penetration of $Na^+$; *i.e.*, that $Na^+$ has a large hydrated radius. If this were so, then $Rb^+$ and $Cs^+$ with hydrated sizes close to that of $K^+$ ought to show similar penetration rates.

The experiments to be reported in this paper were done in order to have a comparison between the rates of penetration of $Na^+$, $K^+$, $Rb^+$, and $Cs^+$, and in order to use such measurements as a basis for the support of a suggestion made previously (3) regarding the mechanism whereby the membrane can discriminate between ions. Because of the physiological importance of $Ca^{++}$, it seemed desirable to include a consideration of the penetration rate for alkaline earth cations, hence the influxes of $Ca^{++}$, $Ba^{++}$, and $Ra^{++}$ have been measured.

### *Methods*

Frog sartorius muscles were carefully dissected, weighed, and placed in Ringer solution with a composition: NaCl 110 mM, KCl 2.5 mM, $CaCl_2$ 1.8 mM. Isotopes of the

* Aided by a grant (B-139) from the National Institute for Neurological Diseases and Blindness, Bethesda.

[1] I am indebted to Dr. R. H. Adrian for some unpublished data on this point.

alkali metal series, $Na^{22}$, $K^{42}$, $Rb^{86}$, and $Cs^{134}$ were added to separate aliquots of Ringer solution in amounts to give about equal counts, and muscles were added to such solutions. The amount of added K, Rb, or Cs was calculated to give a concentration of 2.5 mM so that $[K^+]_o$, $[K^+ + Rb^+]_o$, or $[K^+ + Cs^+]_o$ equalled 5.0 mM. Muscles, usually in pairs, were immersed in these solutions and after 0.25, 0.5, 0.75, 1.0, and 1.5 hours each muscle was removed from radioactive solution, washed 1 minute in inactive Ringer of the same composition as the radioactive, and then placed in a scintillation well counter and counted with a $\gamma$-ray spectrometer. Counting was done with a narrow window width set just at the principal $\gamma$-ray peak of the isotope. As background was somewhat less than 1 C.P.M., counting could be completed in 1 minute and the muscle returned to radioactive Ringer until the next sample time. Influx of the ions involved was calculated on the basis that the extracellular space of the muscle is 15 per cent and fiber water is 70 per cent of the muscle weight less extracellular space. As the efflux of radioactive ions is negligible over the times used for immersion, (with the possible exception of $K^+$ at 1.5 hours), influx was calculated from the initial slope of the uptake curve.

Measurements with divalent cations of the alkaline earth series were made in the following ways. A Ringer solution was prepared to contain either $Ca^{45}$ and $Ba^{133}$ or $Ca^{45}$ and $Ra^{226}$. In either case the $\beta$-counts of $Ca^{45}$ were adjusted to be about 100 times the $\gamma$-counts of $Ba^{++}$ or $Ra^{++}$. This made it possible to count the $Ca^{++}$ with a thin window GM tube with negligible correction for the other isotope, while scintillation spectrometry of the $\gamma$-rays from $Ba^{++}$ or $Ra^{++}$ was entirely uninfluenced by $Ca^{++}$. Radium was obtained as freshly prepared $RaBr_2$ solution and this was boiled to dryness first with HCl and then with distilled water. If this is not done, the strong $\gamma$-emission from Rn masks the 0.19 Mev $\gamma$-ray of Ra. A further complication in working with $Ba^{++}$ is that muscle is highly sensitive to this ion and will undergo continuous twitching in concentrations as dilute as 0.15 mM. This appears to be a direct action of $Ba^{++}$; curarization of the muscles did not prevent such action; and $Ra^{++}$ at 0.3 mM did not exhibit such an action.

Measurements with divalent cations were also complicated by the tendency of such ions to "absorb" on the muscle. This was noticed when a muscle was immersed in $Ca^{45}$-Ringer for 15 minutes and then washed in inactive Ringer for times up to 2 hours. The technique for estimating the influx of divalent cations was to immerse the muscle in radioactive Ringer for times of the order of 2 to 6 hours and then wash in inactive Ringer for 15 to 30 minutes. The muscle could be counted for $\gamma$-radioactivity after various times of wash and these measurements used to estimate when the extracellular space was free of isotope. The muscle was then weighed, dried in an oven, and ashed in platinum at 450°C. The ash was taken up in dilute HCl and counted for $\gamma$-radioactivity; then it was transferred to a planchet, dried, and counted for $\beta$-radioactivity. Influx was estimated by assuming a linear relationship between radioactivity and time as the muscles were rather far from equilibrium. The measured radioactivity was corrected by subtracting from it the radioactivity taken up by a muscle after 15 minutes of immersion and 30 minutes of wash as this was taken to represent ions absorbed somewhere in the muscle. All measurements were made at 20–22°C.

## RESULTS

The measured influxes of the alkali cations are shown in Table I. For $K^+$, $Rb^+$, and $Cs^+$, the rates should be comparable as the concentrations are very close to the same, while for $Na^+$ it is not clear that there is a linear relationship between concentration and flux so that the relative $Na^+$ permeability

TABLE I

*The Influx of Alkali Cations into Frog Muscle*

| Ion | Muscle No. | $C_o$ mM | Influx, millimoles/kg. fiber water hr. | Influx, millimoles/kg. fiber water hr. mM $C_o$ | Relative ion permeability |
|---|---|---|---|---|---|
| $Na^+$ | 40 | 110 | 10.2 | | |
| | 42 | | 9.0 | | |
| | 44 | | 8.8 | | |
| | Mean | | 9.3 | 0.0845 | 0.043 |
| $K^+$ | 20 | 5 | 8.0 | | |
| | 22 | | 10.0 | | |
| | 24 | | 11.0 | | |
| | Mean | | 9.6 | 1.92 | 1.0 |
| $Rb^+$ | 26 | 2.5 | 2.7 | | |
| | 28 | | 2.9 | | |
| | 30 | | 2.3 | | |
| | Mean | | 2.6 | 1.04 | 0.54 |
| $Cs^+$ | 32 | 2.5 | 0.44 | | |
| | 34 | | 0.57 | | |
| | 36 | | 0.60 | | |
| | 38 | | 0.55 | | |
| | Mean | | 0.54 | 0.21 | 0.11 |

will be in error to the extent that its influx per unit concentration deviates from a linear relationship. From the results obtained by Sjodin (4) on the flux-concentration relationships of $Cs^+$ (which is an ion with a permeability only a few times larger than that for $Na^+$) it might be expected that $P_{Na}$ would increase with decrease in concentration. At a concentration of 5 mM $K^+$ the membrane potential of the muscle fiber is not different from that in 2.5 mM so that no correction has been made for an altered electrical driving force acting on the ions. It seems clear from the data that both $Na^+$ and $Cs^+$ have

penetration rates that are low when compared with that of $K^+$, even if the rate for $Na^+$ is subject to some uncertainty.

The uptake of divalent cations is much more difficult to analyze. The reasons for this are several: (*a*) Divalent cations are likely to form insoluble complexes in the extracellular space of the muscle; if the radioactivity cannot be washed away with a time constant similar to that for monovalent cations, one is apt to count such material as intracellular. (*b*) $Ba^{++}$ at low concentrations causes continuous twitching of muscle, while at high concentrations it rapidly produces a maintained contracture. (*c*) Divalent cations are much more likely to react with connective tissue and other protein constituents of muscle not connected with the fiber water. A further difficulty of a technical nature makes the $Ca^{++}$ uptake less reliable and this is the softness of the $Ca^{45}$ $\beta$-radiation. This made it necessary to destroy the muscle in order to have a single point on a kinetic curve, so that uptake kinetics involve much more scatter. For both $Ba^{++}$ and $Ra^{++}$, it is possible to follow the uptake by scintillation counting. In order to correct for the exchange of $Ca^{45}$ with $Ca^{++}$ not in the fiber water, a number of muscles were exposed to $Ca^{45}$ for from 30 to 60 minutes. These were then washed with inactive Ringer of the same composition for the same length of time and the muscles were then ashed and counted. The values obtained for activity were taken as zero-time values since the count increase in the 1st hour of immersion was very much greater than that in a 2nd hour; further there was no difference in the count of muscles immersed either 30 or 60 minutes and then washed for an equal time. There was less difficulty with $Ba^{++}$ and $Ra^{++}$ because these ions could be estimated repeatedly in a muscle by simply washing out the extracellular space for 15 to 30 minutes. As has been mentioned previously, the only ion among those used that induced twitching of the muscles was $Ba^{++}$; in a concentration range from 0.3 to 1 mM the muscles went into rigor in from 1 to 2 hours. This action might be blamed on a "toxic heavy metal" action were it not for the fact that $Ra^{++}$ (which is much heavier, and chemically quite similar) produced no twitching but did, in 5 to 8 hours produce contractures in concentrations of 0.3 mM. In the range of concentrations from 0.1 to 0.3 mM $Ba^{++}$ some muscles did not twitch but it was found safer to work at concentrations of 0.025 to 0.050 mM $Ba^{++}$ if twitching were to be avoided entirely. As the specific activity of $Ba^{133}$ was only about 5 mc./gm. this meant counting muscles with only 50 to 100 C.P.M. Muscles treated with such low concentrations of $Ba^{++}$ were still excitable after 5 hours in contact with $Ba^{++}$ solution.

The results obtained for the uptake of muscles treated with $Ca^{++}$ at 1.8 mM and $Ba^{++}$ at 0.025 mM are shown in Fig. 1. The lines drawn represent best estimates of the rate of increase of radioactivity at times greater than 1 hour. The variability is so great that it is probably not safe to conclude that the apparent rise in radioactivity represents a real increase in intracellular

$Ba^{++}$ or $Ca^{++}$ because the increase in count could represent a slowly exchanging extracellular depot. Moreover, the possibility cannot be excluded that the increase in radioactivity may, in part, represent the precipitation in the extracellular space of some complex between $Ba^{++}$ or $Ca^{++}$ and molecules produced intracellularly by metabolism. On the assumption that the lines drawn in Fig. 1 represent influx, Table II gives numerical values for such influx together with values obtained on a series of 8 muscles using $Ra^{++}$.

When experiments using $Ba^{++}$ were begun, relatively high concentrations (0.3 mM) were used. Because of the continuous twitching and early deterioration

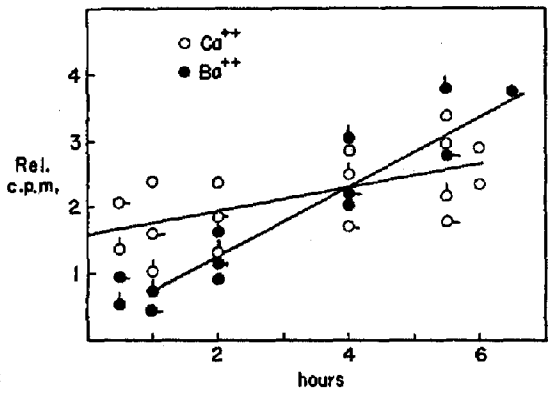


FIG. 1. The ordinate is counts per minute per gram muscle for either isotope; the $Ca^{++}$ counts have been divided by a factor so that they can be represented on the same scale with those for $Ba^{++}$. Each $Ca^{++}$ point represents a single muscle, while lines drawn from the point in the same direction indicate a single muscle on which both $Ba^{++}$ and $Ca^{++}$ were measured. Unmarked $Ba^{++}$ points indicate measurements on a single muscle.

TABLE II

*The Influx of Alkaline Earth Ions into Frog Muscle*

| Ion | Influx, *millimoles/kg. fiber-water hr.* | $C_o$ *mM* | Influx, *millimoles/kg. fiber water hr. mM* $C_o$ | Relative ion permeability |
|---|---|---|---|---|
| $Ca^{++}$ | 0.009 | 1.8 | 0.005 | 1.0 |
| $Ba^{++}$ | 0.0004 | 0.025 | 0.016 | 3.0 |
| $Ra^{++}$ | 0.0015 | 0.30 | 0.005 | 1.0 |

of the muscle, we regarded these experiments as unsatisfactory. They did show, however, that after 1 to 2 hours, while the muscle was fully excitable, the $Ca^{45}$ and $Ba^{133}$ influxes were increased some 3 to 5 times over values obtained with

low $Ba^{++}$ concentrations. As Shanes[2] finds that the extra uptake of $Ca^{++}$ per impulse is some 30 times the extra uptake of $Ca^{++}$ per impulse in squid axon (5) the extra $Ca^{++}$ uptake found is understandable. The increment in $Ba^{++}$ influx might be thought to be due to the same mechanism that increases the $Ca^{++}$ influx, or, it might be argued, that the increased $Ba^{++}$ concentration has increased the permeability of the membrane for this ion. It is also possible that the great increase in metabolism in muscle that is brought about by contraction leads to an increased precipitation of both ions in the extracellular space.[3]

## DISCUSSION

The mode of transfer of an ion from aqueous solution to the membrane can only be considered with reference to specific models of the membrane, and some of these are examined in the following discussion. One of the principal models of the membrane has been that of an ion exchange system. At the outset it must be noted that the membrane of the muscle cell differs from any ion exchange system in several important respects: the membrane is somewhat more permeable to $Cl^-$ than it is to $K^+$, but this difference is estimated to be of the order of 3:1 (6) while ion exchange systems differ in discriminating strongly between ions of opposite charge. The membrane passes $K^+$ much more readily than $Cs^+$, while ion exchange membranes generally are more permeable to $Cs^+$ than to $K^+$, or else do not distinguish between these ions. Ion exchange systems discriminate only poorly between ions such as $Na^+$ and $K^+$, while cell membranes exhibit a high order of selectivity. The evidence presently available, while insufficient to reject conclusively ion exchange mechanisms as involved in the membrane of the muscle cell, by no means requires the use of such a model, and it seems an unwarranted complication to postulate an ion exchange system unless, as is the case for the red blood cell, anion: cation permeation rates differ by a factor of $10^6$ (7). A series of ion exchange systems that show very high selectivities with respect to ions such as $Na^+$ and $Cs^+$ are described by Barrer and Falconer (8). These are inorganic crystals with a pore system. It is presumably the pore size that effects the discrimination here. In-

---

[2] Personal communication.

[3] I am indebted to Dr. A. M. Shanes for pointing out that some of the $Ca^{++}$ that we call "adsorbed" because it appears in the first half-hour of immersion may actually be due to an insufficient clearing of the extracellular space of $Ca^{45}$. This is so because while preliminary experiments were done with 30 to 40 mg. muscles, later measurements were with 60 to 80 mg. muscles when times longer than an hour may be required for the removal of extracellular $Ca^{45}$. If such a correction is necessary, its application would raise the absolute values of influx without affecting the ratio: $P_{Ba}P_{Ca}$

deed, the authors propose a dielectric model for the exchange process because the energies of exchange are so low.

A second model of the membrane is that of a homogeneous lipide film. In such a model, ions present externally must dissolve in the membrane phase and diffuse to the other side. Without any information on the chemical composition of the phase it is impossible to make statements regarding its specificity for ions but in view of the general difficulty in distinguishing chemically between $K^+$ and $Cs^+$, the model is not a reassuring one. A further difficulty is that such a lipide phase must be expected to have a dielectric constant less than 25 and therefore penetration could only take place by ion pair formation. The existence of such an effect ought to make the movement of ions across the phase independent of the electric potential difference while the evidence is good that much ion movement is not independent of the membrane potential (9).

The most acceptable model of the membrane is one composed of aqueous pores with intervening ion-impermeable areas; this is indicated both by the presence of a "long pore" effect (10) and by the finding of a difference between the osmotic and diffusion permeability of a variety of cells (11). It is convenient to distinguish between two types of pore membranes: (*a*) the pores are greater than about 10 A in radius, and (*b*) the pores are much less than 10 A in radius. The reason for this distinction will be discussed further below, at this point it might be noted that pores larger than 10 A will allow an ion to pass with enough water to satisfy the energy requirements for hydration. A membrane with large pores will not, therefore, discriminate between ions unless one invokes chemical interactions between the ion and the pore walls.

The crystal radius of $K^+$ is 1.33 A, and the hydration energy of the ion is about 75 kcal./mole. From the magnitude of the hydration energy it is clear that in aqueous solutions at room temperature an unhydrated $K^+$ has no physical reality. A parameter that is sometimes considered is the "hydrated ion radius." This is obtained by measuring the ion mobility in aqueous solution and then calculating the radius of a sphere that would move under the driving force of the applied electric field with a velocity equal to the measured ion mobility, assuming Stokes's law. The validity of this operation is questionable: Stokes's law is applicable only to particles that are large compared with the solvent, and no allowance is made for solvent exchange which is a process whereby the ion can advance through the solvent by adding hydration in the direction of travel. A further difficulty is that the "hydrated ion radii" for $Rb^+$ and $Cs^+$ are practically identical with that for $K^+$, yet the ions behave in the membrane as if their sizes were different.

A different approach to the problem of ion hydration is to ask how many water molecules must surround an ion in order to have its demand for hydration largely satisfied. A calculation (3) shows that an ion with three complete shells of water molecules has its electric charge largely shielded, and hence has little

further demand for hydration. The radius of such an ion can be computed by adding to the crystal radius of $K^+$, 3 times the diameter of the water molecule (2.72 A) or ($1.33 + 3 \times 2.72 = 9.49$ A), and it is for this reason that the value of 10 A was listed as a pore size where an ion could penetrate under conditions similar to diffusion in aqueous solution. A discrimination based on ion size could be made by supposing that membrane pore sizes are rather less than 10 A in radius and that the residual demand for hydration that an ion may have must be satisfied by substituting for water molecules the material of the membrane pore. If we consider only right cylindrical pores, the foregoing assumption simplifies to a consideration of the following cases: the ion has 2, 1, or 0 complete shells of water molecules, as these give ions with circular profiles. To discuss the mechanism of ion penetration, it is not necessary to decide upon a particular hydration configuration. In what follows, however, the case of an ion with one complete shell of water molecules is assumed. The arguments apply equally to the other cases, and the reason for taking this particular case is that the sizes of pores obtained are such that they could be used for molecule penetration as well. Thus, a rapidly penetrating molecule such as benzene has a radius of about 3.5 A and a poorly penetrating molecule such as sucrose has a radius of about 4.8 A.

If an ion is to penetrate through a membrane composed of small pores, it must replace the water molecules that are serving as hydration with other molecules (the pore walls) which will serve this function. It has been suggested (12) that $Na^+$ may be kept out of the membrane because its greater energy of hydration holds it in the aqueous phase while $K^+$, $Rb^+$, and $Cs^+$ are selectively passed on the basis of their crystal radius. This does not seem at all likely on the basis of energy considerations. The hydration energies of $Na^+$ and $K^+$ are about 95 and 75 kcal./mole, and as these energies are of the order of the strength of stable chemical bonds, it seems necessary to conclude that the observed influxes of these ions cannot be due to the escape of the ions from their hydration. Even if we consider the ion with a complete shell of water molecules, the hydration energies are too large for any reasonable number of ions with the requisite energy to exist at room temperature. It seems, therefore, necessary to suppose that the energy diagram shown in Fig. 2 has a tunnel which allows ions to pass from aqueous solution to pores under certain conditions. These would appear to be that the ion can largely replace its hydration beyond the first shell with a solvation of similar magnitude obtained from the pore wall. For the solvation from the pore wall to be at all effective it must fit the ion closely and the conclusion is, therefore, that ions will only penetrate into pores that they fit closely. If the pore is too small, the ion will be excluded for steric reasons; if the pore is too large, the ion will be excluded because no tunnel exists in the hydration energy diagram.

On the molecular scale it seems unlikely that pores would be monotonic in

size, and a more reasonable assumption is that they are distributed in size according to a Gaussian curve. If the mechanism for penetration that has been proposed is correct, it ought to be possible to measure some of the parameters of this curve by taking the relative permeabilities for the alkali cations and representing these as areas, along an axis for ion radius. The ion radius is taken as crystal radius + 2.72 A (although it could equally well be taken as crystal radius alone). A Gaussian curve is then constructed to fit the experimental measurements and this is shown in Fig. 3. In order to represent ion size as an

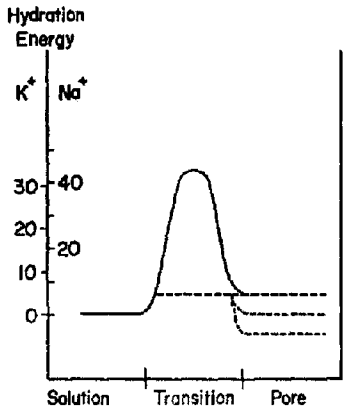


FIG. 2. The ordinate is the hydration energy of a singly hydrated $Na^+$ or $K^+$ and the abscissa is the position of the ion. At the left, the ion is in solution and is fully hydrated; in the center it is in a transition region just at the entrance to a pore and at the right it is in a pore. The dashed line in the transition region indicates a "tunnel" in the energy diagram, while the dashed lines in the pore region indicate levels of hydration energy depending upon whether the polarizability of the pore wall is less than, equal to, or greater than that of the aqueous solution.

area, an arbitrarily chosen tolerance for close fit between an ion and a pore must be made. In the curve shown this is $\pm 0.05$ A. If the tolerance is made twice this value ($\pm 0.1$ A) the permeability data are not greatly altered. Fig. 3 has relative permeabilities: $Na^+ = 0.03$, $K^+ = 1$, $Rb^+ = 0.64$, and $Cs^+ = 0.08$, while with twice the tolerance the values are 0.08:1:0.47:0.15. No great effort has been made to make the extremes of the distribution fit the experimental data closely because, since the standard deviation and the fit tolerance can be adjusted independently, the curve can only serve as a rough guide to the dispersion in the membrane. It is clear, however, that if the mode of the dispersion were shifted slightly to the right, $Rb^+$ would become more permeable than $K^+$, as is found with invertebrate fibers.

The usefulness of this method of analysis would be greatly enhanced if it

could be shown to be generally applicable to the penetration of all ions. Lithium ion is a particular case and one may ask what the predictions are here. The difficulty is that its crystal radius (0.65 A) is so small compared with that of $Na^+$ (0.95) and hence the surface areas of the ions vary as 1:3. The structure of the first hydration shell must be very different for the two ions. Whether this leads to an ion that is somewhat smaller or somewhat larger than $Na^+$ or to a mixture of hydration configurations cannot be ascertained. The same considerations apply to $Mg^{++}$ which has the same crystal radius but there are additional complications. The anions $F^-$, $Cl^-$, $Br^-$, and $I^-$ would appear to offer more promise of analysis but there are complications here too. $F^-$ has the same crystal

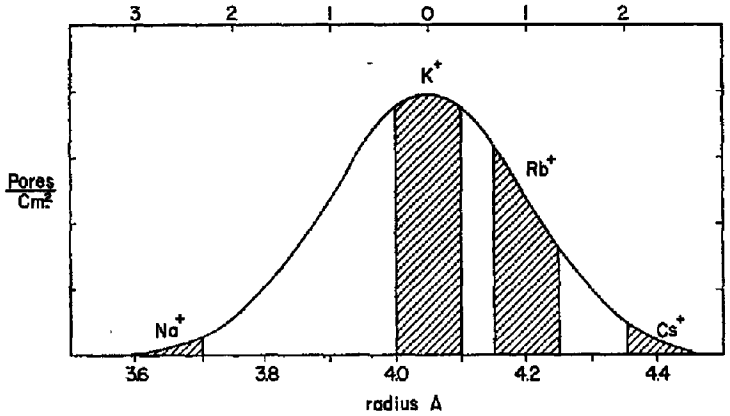


FIG. 3. The shaded areas represent the number of pores in the distribution that fit an ion of a particular size to within ±0.05 A. The curve is drawn with 1 standard deviation equal to 0.15 A, and with a mode at 4.05 A. The ordinate is the number of pores per unit area of membrane and the abscissa is the radius of the ion (crystal radius + 2.72 A). At the top of the diagram, standard deviation is shown as abscissa.

radius as $K^+$ but reacts with $Ca^{++}$. Chloride ion, with a crystal radius of 1.81 A, ought to yield a very large hydrated ion (larger than $Cs^+$) were it not for the fact that there is known to be a relatively large contraction of the hydration shell because water molecules around an anion are oriented in the opposite direction to that around a cation and this leads to a more efficient packing. Allowance for the contraction would make $Cl^-$ about the size of $Rb^+$, but it would have a different shape because of the altered orientation of the water molecules. It is also likely that the partition coefficients for cations and anions to the membrane are different so that it is clear that we cannot relate cation and anion penetration rates on any absolute scale. Relative values for anion penetration rates ought, however, to be obtainable by reference to pore size distribution and it seems clear (13) that a large anion such as $I^-$ is less permeable than $Cl^-$.

Because some divalent cations (especially $Ca^{++}$) have important physiological functions it seems especially useful to attempt to analyze their physical properties. The crystal radius of $Ca^{++}$ is almost exactly that of $Na^+$ so that if the preceding discussion is valid $Ca^{++}$ must be expected to compete for membrane pores with $Na^+$. This fact undermines the analysis of membrane pore dispersion that has been made as it has been assumed that the rate of $Na^+$ penetration reflected the number of $Na^+$-sized pores in the distribution. The evidence that $Ca^{++}$ behaves as does $Na^+$ is rather good. It has been shown that there is an extra influx of $Ca^{++}$ per impulse in squid axon (5) that is proportional to $[Ca^{++}]_o$, and that there is a need for the active extrusion of $Ca^{++}$ from muscle (14). That $Ca^{++}$ behaves as a poorly penetrating $Na^+$ is suggested both from measurements of its influx (5) and from the alteration it makes in the squid axon under voltage clamp conditions (15). There is, however, the additional complication that low $Ca^{++}$ concentrations appear to increase membrane conductance in the steady-state and this increase in conductance is in large part due to an increased $P_K$ of the membrane. Indeed, the experimental information available suggests that in addition to competing with $Na^+$ for pores in the membrane, $Ca^{++}$ may influence the distribution of pore sizes. A similar explanation appears necessary to account for the effects of replacing $Na^+$ in Ringer solution with $Rb^+$ or $Cs^+$ (4) as the permeabilities of these ions are affected by concentration.

Barium ion was selected for study on the basis that its crystal radius is very close to that of $K^+$. Accordingly we might infer that it would behave as a slowly moving $K^+$, and that it would compete with $K^+$ for transfer across the membrane. The results obtained, however, suggest that there is little difference in the rate of penetration of $Ba^{++}$ and $Ca^{++}$, certainly nothing like the difference in rate between $K^+$ and $Na^+$. This finding might mean that the hypothesis used to explain the penetration rates for the alkali cations is wrong, or that the rate-limiting step for divalent cation penetration is different from that for the alkali cations. This would be true if divalent cations moved very slowly through membrane pores and if this rate could not be increased by more frequent collisions of $Ba^{++}$ at the outside of the membrane. On this basis increases in influx with increases in $C_o$ require that the divalent cation occupy more pores in the distribution as its concentration is increased. With a finite number of pores, this process cannot go on indefinitely so that the saturation of $Ca^{++}$ influx found in squid axon at $[Ca^{++}]_o$ equal to 40 mM (5) is understandable on this basis. In the present experiments the relative concentrations of Ca:Ba were usually 70:1 and the influx of $Ba^{++}$ was somewhat greater per unit concentration, which can be interpreted as indicating that $Ba^{++}$ is a good competitor for pores. We have no information as to the concentration at which the $Ca^{++}$ influx saturates in frog muscle.

Measurements with $Ra^{++}$ which has a crystal radius (extrapolated) that is not greatly different from that of $Cs^+$ show that its rate of penetration is inferior to

that of $Ba^{++}$ and, allowing for the greater experimental uncertainty, the value for $P_{Rb}/P_{Ba}$ is not different from that for $P_K/P_{Cs}$. A further point of similarity is that the depolarization produced by $Cs^+$ is small, compared with that of $K^+$; the excitatory action of $Ba^{++}$ is not shown by $Ra^{++}$ at concentrations 3 times as high.

The diagram shown in Fig. 4 has been made to indicate the sort of correction that may be necessary to represent properly membrane pore distributions. It reflects the following theoretical assumptions: (*a*) the partition coefficient for divalent cations may be expected to be larger than that for the alkali cations, (*b*) the mobility for divalent cations may be expected to be much smaller, and

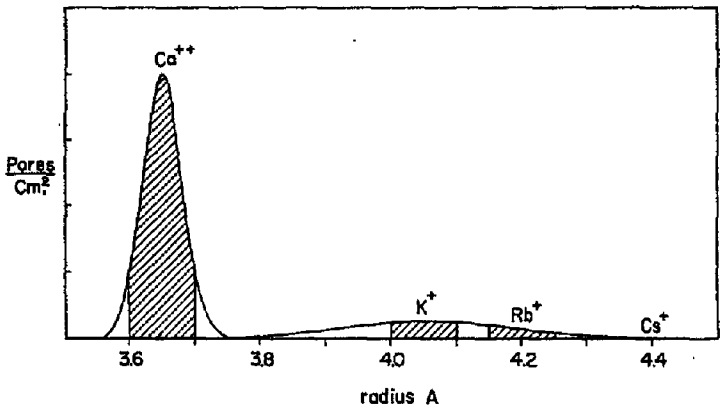


FIG. 4. A membrane pore dispersion diagram as in Fig. 3 but drawn on the assumption that the majority of the pores are occupied by poorly penetrating divalent cations. On the scale used neither the areas for $Cs^+$ nor $Na^+$ penetration are visible.

(*c*) the membrane pores are not rigid, but are deformable; the large energies of hydration (or solvation) that can be exerted by ions allow them to exert deformation. The justification for assumption (*a*) is that the physiological effects of alkaline earth cations are largely confined to a range of concentration that is small compared with Ringer concentrations and, these effects take place with little or no change in membrane potential. Assumption (*b*) is required in order to reconcile the very low permeability of $Ca^{++}$ with (*a*), while (*c*) appears necessary to explain changes in permeability with changes in the concentration of ions outside the cell.

It seems difficult to escape the conclusion that the marked effects of $Ba^{++}$ on frog sartorius are exceptional rather than general. Thus, Fatt and Ginsborg (16) find it possible to use 160 mM $Ba^{++}$ on crustacean muscle. This concentration may be expected to be very effective in changing the membrane pore distribution. We have, in some unpublished experiments, shown that squid axon

is unaffected by concentrations of $Ba^{++}$ of the order of 1 to 5 mM, although conduction is blocked by 10 to 20 mM. Similar findings have been reported for frog sciatic nerve (17).

## REFERENCES


1. Conway, E. J., and Moore, P. T., *Nature*, 1945, **156**, 170.
2. Hodgkin, A. L., *Proc. Roy. Soc. London, Series B*, 1957, **148**, 1.
3. Mullins, L. J., *Am. Inst. Biol. Sc. Symp.* No. 1, 1956.
4. Sjodin, R. A., *J. Gen. Physiol.*, 1959, **42**, in press.
5. Hodgkin, A. L., and Keynes, R. D. *J. Physiol.*, 1957, **138**, 253.
6. Hodgkin, A. L., and Horowicz, P., *J. Physiol.*, 1957, **137**, 30*P*.
7. Tosteson, D. C., *in* Electrolytes in Biological Systems, (A. M. Shanes, editor), Washington, D. C., American Physiological Society, 1955, 123.
8. Barrer, R. M., and Falconer, J. D., *Proc. Roy. Soc. London, Series A*, 1956, **236**, 227.
9. Hodgkin, A. L., Huxley, A. F., and Katz, B., *J. Physiol.*, 1952, **116**, 424.
10. Hodgkin, A. L., and Keynes, R. D., *J. Physiol.* 1955, **128**, 61.
11. Ussing, H. H., *Symp. Colston Research Soc.*, 1954, **7**, 33.
12. Shanes, A. M., *Pharmacol. Rev.*, 1958, **10**, 59.
13. Padsha, S. M., *J. Physiol.*, 1957, **137**, 26*P*.
14. Gilbert, D. L., and Fenn, W. O., *J. Gen. Physiol.*, 1957, **40**, 393.
15. Frankenhaeuser, B., and Hodgkin, A. L., *J. Physiol.*, 1957, **137**, 218.
16. Fatt, P., and Ginsborg, B. L., *J. Physiol.*, 1958, **142**, 516.
17. Lorente de No, R., and Feng, T. P., *J. Cell. and Comp. Physiol.*, 1946, **28**, 397.