Movement of Inorganic Ions across the Membrane of Striated Muscle

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The unidirectional fluxes of sodium, chloride, and potassium across the membrane of striated muscle cells are described under a number of experimental conditions. The results are compared with the predicted behavior of simple membranes. With the possible exception of chloride, it is concluded that the movements of these ions cannot be described without postulating variable permeabilities or a variable number of 'carriers' or 'sites' in the membrane.

It is now widely accepted that many substances cross cellular membranes by special mechanisms. The movement of an ion into a region of greater electrochemical potential plainly requires a mechanism which can be linked to a source of energy, but even movements down gradients of chemical or electrochemical potential may require special mechanisms if they are to take place with sufficient speed. The facilitated diffusion of glucose across the wall of the red cell is an example. It is becoming increasingly clear that the movements of many inorganic ions across the surface membrane of muscle cells cannot be explained in terms of diffusional and electrical forces only.

A muscle cell is thought to maintain its internal ionic composition by pumping out the sodium that enters the cell by diffusion down the sodium concentration gradient. Linked to the driven outward sodium movement, but not necessarily in a 1 to 1 manner, there is an inward potassium movement. The potential across the cell membrane will have a value such that the potassium lost from the cell by movement down its concentration gradient is balanced by the active uptake. Since the electrochemical potential of chloride appears to be equal on either side of the membrane, there is no need to postulate any energy-consuming mechanism influencing chloride movements. This description is probably valid as a general outline of the situation, though there is disagreement on the occurrence of linked sodium and potassium movements. A number of authors do not agree that a sodium pump is necessary and prefer to consider that the high, internal potassium concentration of nerve and muscle cells is the result of binding on selective sites distributed throughout the substance of the cell. In what follows I will assume that the internal ions behave as if they were in free solution and that the rate-limiting step for ionic movements resides within the plasma membrane. But even making this assumption we are faced with a large number of unanswered questions about how ions move in the membrane.

For an ion which is acted on only by the forces of thermal agitation and electrical potential, and which does not compete for chemical combination with a molecule in the membrane, the ratio of the outward flux \( M_{\text{out}} \) to the inward flux \( M_{\text{in}} \) is given by

\[
\frac{M_{\text{out}}}{M_{\text{in}}} = \frac{C_{\text{in}}}{C_{\text{out}}} \exp \left( \frac{zEF}{RT} \right)
\]

where \( E \) is the internal potential, \( z \) the algebraic valency, and \( C_{\text{in}} \) and \( C_{\text{out}} \) the internal and external concentration. The activity coefficients on the inside and outside are assumed to be equal. This equation was first derived by Bohm (1897) and has been discussed by Ussing, Teorell, and Hodgkin among others. The equation is independent of variations within the membrane of potential, concentration, activity coefficient, and mobility. It does not, however, tell us the magni-
tude of either flux. To calculate the individual fluxes and the way in which they vary with membrane potential and concentration, one has to make assumptions about the way in which the potential, activity, and mobility vary within the membrane.

The assumptions which lead to the most manageable flux equations are that the mobility of the ion is constant across the membrane and that the potential in the membrane varies linearly. This is generally called the constant-field assumption and was first made by Goldman.\textsuperscript{10} The equations for the two fluxes of a monovalent cation are

\begin{equation}
M_{\text{out}} = P \frac{\text{EF}}{RT} \frac{C_{\text{in}} \exp \left( \frac{\text{EF}}{RT} \right)}{\exp \left( \frac{\text{EF}}{RT} \right) - 1} \tag{2}
\end{equation}

\begin{equation}
M_{\text{in}} = P \frac{\text{EF}}{RT} \frac{C_{\text{out}} \exp \left( \frac{\text{EF}}{RT} \right)}{\exp \left( \frac{\text{EF}}{RT} \right) - 1} \tag{3}
\end{equation}

where \(P\) is a permeability constant of the form \(\frac{u \beta RT}{aF}\) and has the dimensions of cm./sec.\textsuperscript{11} The thickness of the membrane is \(a\); \(u\) is the mobility in the membrane, and \(\beta\) is the partition coefficient for the ion between the membrane and the aqueous solution. The assumption of a constant field has been widely used and as widely criticized. Other equations for the fluxes have been based on other assumptions about the potential, concentration gradient, and activity within the membrane. Teorell has shown that in membranes with fixed charges the potential gradient is not linear, and he has derived flux equations for fixed charge membranes.\textsuperscript{12} Linderholm\textsuperscript{13} has treated the case of a membrane with no fixed charges in which the activity coefficient varies with the concentration. But these equations have in common with the constant-field equations the fact that the flux ratio obeys equation 1. They all predict that so long as the membrane potential remains unchanged and the total concentrations on each side of the membrane are the same, then the efflux of a particular ion will depend on the internal concentration of that ion, the influx of the same ion will depend on its external concentration, and both fluxes will be directly proportional to the mobility of the ion in the membrane. This fundamental similarity of the flux equations arises because they are all based on the assumption that the chance of any one ion crossing the membrane is uninfluenced by the presence of any other ion inside or outside; in other words that the ions cross the membrane independently of each other.

The flux ratio equation has only been tested over a wide range of potential and external concentration in the case of giant axons from the cuttlefish \textit{Sepia}. Hodgkin and Keynes\textsuperscript{14} measured the two fluxes in axons poisoned with DNP to eliminate pumped-inward potassium movement. They found that the logarithm of the flux ratio varied more steeply with potential than was predicted by equation 1 for a monovalent cation. They explained their results by supposing that potassium crossed the membrane along a chain of negatively charged sites or through long narrow pores. The essential feature of both explanations is that in any one chain of sites or in any one tube, ions move in single file and can not pass one another.

Ussing\textsuperscript{15} has suggested a mechanism which will account for deviations from the flux ratio equation in the opposite direction—for a flux ratio which is less sensitive to changes in potential and concentration than predicted by equation 1. If a carrier molecule exists in the membrane which is unable to cross from one side to the other unless combined with a particular kind of ion, there will be equal and opposite movements of this ion carried across the membrane by the movement of the ion-carrier complex. If the carrier is unable to combine with any other ion and unable to cross the membrane uncombined, the magnitude of the equal carrier-born influx and efflux will be independent of the membrane potential and dependent on the concentrations on either side of the membrane. Since the influx must always equal
the efflux there can be no net movement of an ion by such a mechanism.

Keynes and Swan have shown that the efflux of $^{24}$Na from a sartorius muscle into an ordinary frog Ringer's solution is reduced by more than 50 per cent when the sodium in the Ringer's fluid is replaced by lithium or choline. They also found that the removal of extracellular potassium reduced the efflux of $^{24}$Na. Removal of both sodium and potassium produced a larger reduction of efflux than the removal of either alone. They studied the relation between the external sodium concentration and the efflux of sodium, which could be reasonably well fitted by an equation of the Michaelis-Menten type with a half-saturation value for the external sodium concentration of 38 mM (fig. 1, from Keynes and Swan, 1959).

Because the membrane potential of the muscle fiber at rest (inside $-92$ mV) is so far removed from the sodium equilibrium potential (inside $+50$ mV) fluxes of sodium resulting from thermal and electrical forces alone would be very unequal. The efflux would only be 0.003 of the influx. Experimentally the influx and efflux are about equal for a muscle in Ringer's fluid and both are about 10 pmoles/cm.$^2$/sec. If half the sodium efflux is by an exchange diffusion mechanism, half the sodium influx is by the same mechanism, and 5 pmoles/cm.$^2$/sec. represents the passive influx which must be balanced by the pump. Applying the constant-field equation for the influx (equation 3), a flux of 5 pmoles/cm.$^2$/sec. corresponds to a $P_{Na}$ of $0.01 \times 10^{-6}$ cm.$^2$/sec., which is in satisfactory agreement with estimates of $P_{Na}$ by electrical methods.

Keynes and Swan have also shown that in circumstances where the internal sodium

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**Figure 1**

The relative magnitude of the efflux of $^{24}$Na when the external sodium is varied by substituting lithium. (From Keynes and Swann, 1959.)
might be expected to have risen, the exchange diffusion component of the sodium efflux diminishes and the potassium coupled component increases. They make the attractive suggestion that the Na-Na exchange carrier can be changed into a carrier, which, when coupled to a source of energy, moves sodium out and potassium in. The proportion of the carrier existing in either form might depend on the internal sodium concentration. Such a mechanism would tend to keep the internal sodium concentration at a fixed level. In these terms exchange diffusion of sodium can be thought of as what the sodium pump does when it is not required to move sodium and potassium up their electrochemical gradients.

It is not surprising that special mechanisms are needed to explain the movements of sodium, because muscle cells, and indeed most cells, exist in a steady state with the electrochemical potential of sodium inside and outside far from equal. In frog muscle the distribution of the chloride ion seems to be what would be expected if chloride were acted on by thermal and electrical forces only. Provided sufficient time is given to reach equilibrium, the ratio of the internal and external concentrations of chloride is given by the equation

\[ \frac{C_{in}}{C_{out}} = \exp \left( \frac{EF}{RT} \right) \]

but even so it is not yet clear whether the movements of chloride in each direction are independent. Hodgkin and Horowicz and Adrian have both suggested that chloride may cross the muscle membrane by single-file mechanism of the kind postulated for the passive potassium fluxes of Sepia. From experiments on isolated muscle fibers from the semitendinosus of the frog, Hodgkin and Horowicz concluded that the net movement of chloride under a wide variety of conditions could be predicted by the constant-field equation with a mean value for \( P_{Cl} \) of \( 4 \times 10^{-6} \) cm./sec. When the influx and efflux of chloride are equal, the constant-field equation takes the form,

\[ M_{in} = M_{out} = \frac{C_{in}}{C_{out} - C_{1}} \ln \frac{C_{in}}{C_{1}} \]  

Setting \( P_{Cl} = 4 \times 10^{-6} \) cm./sec., this equation predicts that a muscle in equilibrium with a Ringer's solution which contains 50 mM/1-NaCl and 120 m-mole/L-NaCl will have a chloride efflux of 330 pmole/cm²/sec. In equilibrium with a similar solution with 100 m-mole/L-KCl, the efflux would be 540 pmole/cm²/sec. The effluxes under both conditions have been measured and are found to be respectively 50 and 200 pmole/cm²/sec. The number of observations is not large, but if the discrepancy is a real one it is of the kind that would be expected if there were a single-file mechanism. Adrian and Freygang (in press) have recently made conductance measurements on fibers in the sartorius muscle. In the 100 mM KCl solution referred to above they estimated \( P_{Cl} \) to be \( 2.2 \times 10^{-6} \) cm./sec., which would go some way to resolving the discrepancy, since the fluxes were measured on sartorius muscle. Their experiments also suggest a fairly wide variation of \( P_{Cl} \) from one muscle to another.

Replacement of the external chloride with a number of anions, for instance \( NO_3^- \), \( I^- \), \( CNS^- \), \( ClO_4^- \), reduces the chloride efflux. In the case of nitrate, 100 per cent replacement of the external chloride halves the efflux of \( 36Cl \). Figure 2 shows the reverse effect, that the efflux of \( 36Cl \) increases when chloride replaces external nitrate. When nitrate replaces chloride the membrane resistance rises substantially, but there appears to be little or no alteration in the membrane potential. Two conclusions can be drawn; that chloride in the presence of nitrate has about the same permeability as nitrate; and that the component of the efflux which disappears when nitrate replaces chloride is not an exchange diffusion flux. If half the chloride efflux were an exchange diffusion flux, there should be no alteration of membrane resistance when nitrate replaces chloride. It is reasonable to conclude that the presence of nitrate alters the permeability to chloride, that is, it alters the ease with which chloride moves within the membrane, or the concentration of chloride within the membrane, or both.

If the chloride movements are independent
but at the same time involve special carriers or pores, these mechanisms cannot be near saturation. For nitrate to affect the chloride flux it would have to occupy a substantial proportion of the carrier molecules or pores. This would be possible if the affinity of the carrier were much greater for nitrate than for chloride, but since the permeabilities of nitrate and chloride are comparable, the mobility of the nitrate-carrier complex would have to be much less than the mobility of the chloride-carrier complex. If the movements of chloride are not independent, special assumptions are needed to explain the fact that net movement appears to fit the constant-field equation. At the moment there is insufficient experimental evidence.

In muscle as opposed to nerve, there is some evidence that there are two channels for the movement of potassium into and out of the fiber. Both channels are capable of carrying a current of potassium ions, but they appear to rectify in opposite directions. There certainly exists in muscle a mechanism similar to the nerve mechanism and responsible for the rapid falling phase of the action potential (delayed rectification). This mechanism has not been studied in detail in muscle, because so far it has not proved possible to apply voltage-clamp techniques to muscle fibers capable of conducting action potentials. In high potassium most of the potassium movement appears to be by a channel which presents a high resistance when the net movement is in an outward direction (anomalous rectification). Katz showed that when the internal and external potassium concentration were made equal and there was no other ion which could carry current, the membrane showed a remarkable asymmetry, having a low resistance for inward current and a high resistance for outward current. Figure 3 shows the same kind of current-voltage relation obtained from an isolated muscle fiber in a sulfate solution with an external potassium concentration of 100 mM.

While the possibility exists that the passage...
of anions can be explained by a fixed number of specialized membrane sites, whether pores or carriers, a similar mechanism with a fixed number of sites is very much harder to visualize in the case of these passive potassium movements. A system with a fixed quantity of carrier or a fixed number of pores which interact only with potassium could not show the asymmetric current voltage relation of figure 3. Hypotheses involving multiply-charged carriers can be made to rectify in this way but only if the total quantity of carrier is allowed to vary. Equally an equation of the constant-field type with a constant permeability coefficient will not describe the behavior in figure 3, though, by appropriately varying the permeability coefficient with potential, this or indeed any other current-voltage relation can be imitated. The validity or usefulness of such arbitrary variation of the permeability "constant" has given rise to a good deal of discussion.23, 24, 25

The potassium current can be considered as the difference between the influx and efflux of potassium when these are altered by altering the membrane potential. It is therefore of interest to study the two fluxes separately under conditions of net potassium movement. It is difficult to alter the membrane potential of muscle by electrical methods during the course of a tracer experiment, but one may do so by making use of situations where the chloride permeability is much greater than the potassium permeability, and the membrane potential therefore controlled by the chloride concentration ratio.17 Muscles were loaded with extra internal potassium and chloride by putting them into a Ringer's solution with a KCl concentration of 100 mM and a NaCl concentration of 120 mM. They reach a steady state in this solution in about 3 hours with an extra 100 mM/l. of both potassium and chloride internally. If the internal potassium has been labeled with 42K, the steady state efflux into the high KCl solution is found to be about 100 pmole/cm.2/sec. Figure 4 shows the effect on the membrane potential and the rate constant for the loss of 42K of replacing the external chloride

with sulfate but leaving the external potassium concentration unaltered at 100 mM. The solutions were arranged so that no immediate movement of water takes place when they are changed. The inside potential alters suddenly from −20 mV to +50 mV. In this situation there should be net outward movements of both potassium and chloride, and they can be shown to occur by direct chemical analysis.26 But the efflux of potassium drops. Since there is a net outward movement the influx must also have dropped. One may say then that with constant outward movement the influx must have been increased efflux, if the permeability were constant.

If after a similar period in the high KCl
solution, a muscle is transferred to a solution with the same chloride concentration but with sodium, lithium, or choline replacing most or all of the potassium, the potential remains unaltered but the efflux of \(^{42}\text{K}\) drops by a factor of 10 or more (fig. 5). Preliminary experiments indicate that the influx drops by more than would be expected if it depended only on the external potassium concentration. The effect of removing external potassium on the efflux of potassium is reminiscent of the exchange diffusion of Na, but the mechanism of the potassium effect cannot be the same because, if only one tenth of the steady state flux were capable of carrying current, the potassium conductance of the membrane in the high-KCl solution would be very small. Conductance measurements in the high-KCl solutions are consistent with a flux of about 100 pmole/cm.\(^2/\)sec.\(^2\). Harris and Sjodin\(^{24}\) have suggested that, in circumstances where the influx and efflux of potassium are equal, all transfer of isotope takes place by an exchange diffusion mechanism, with a fixed number of potassium-saturated sites in an outer region of the cell. A difficulty of such a scheme is that the potassium conductance must be zero when the membrane potential equals the potassium equilibrium potential and the influx and efflux are equal.

Sjodin\(^{27}\) suggested that rubidium and caesium alter the permeability of the muscle membrane to potassium, and he has shown recently\(^{25}\) that rubidium and caesium reduce the uptake of \(^{42}\text{K}\) by muscle. To account for these effects, he has suggested that rubidium, caesium, and potassium compete for a fixed number of sites in the membrane which are not necessarily saturated. In the absence of a competing ion, the uptake of each ion as a function of its external concentration is supposed to be given by the equation

\[
M_{\text{in}} = P_1N\frac{k_1C_{\text{(out)}}}{k_1C_{\text{(out)}} + 1}
\]  

(5)

N is the number of sites in the cell surface; \(P_1\) and \(k_1\) are a pair of constants which Sjodin has compared to the mobility and partition terms in the constant-field permeability coefficient. The values of \(P_1\) and \(k_1\) for each of the three ions are obtained by fitting the uptake rates of \(^{42}\text{K}, ^{86}\text{Rb},\) and \(^{137}\text{Cs}\) at various external concentrations to the above equation. When two competing ion species are present, the uptake of one of them is supposed to be given by the equation

\[
M_{\text{in}} = P_1N\frac{k_1C_{\text{(out)}}}{k'C_{\text{(out)}} + k_1C_{\text{(out)}} + 1}
\]  

(6)

where \(k'\) and \(C'_{\text{(out)}}\) are the appropriate constant and the external concentration of the competing ion. He has achieved an impressive agreement between the theoretical and experimental uptakes under a variety of conditions. However, the agreement must be to some extent a coincidence since it is a feature of Sjodin’s model that the influxes are independent of the membrane potential, and we have

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Figure 6
The rate constant for the loss of $^{42}$K from a KCl-loaded muscle into solutions with the same Cl concentration but varying K concentrations. Na replaced K.

Figure 7
The rate constant for the loss of $^{42}$K from a KCl-loaded muscle into solutions with 100 mM K or with no K and varying Rb concentration. The Cl concentration was the same throughout the experiment. The total cation concentration was maintained with Na.
seen that in some circumstances this is not so. The efflux of an ion in Sjodin’s model is, however, supposed to depend on the membrane potential as well as on the internal concentration, but the potential dependence predicted is in the opposite direction to that found experimentally (fig. 4).

By an extension of the kind of experiment shown in figure 5, one can determine the relation between the efflux of $^{42}$K and the external potassium concentration at substantially constant membrane potential. Figures 6 and 7 show two such experiments, and figure 8 shows the mean effluxes from a number of such experiments plotted against the external concentration of either potassium or rubidium. The effluxes are expressed as a percentage of the efflux into the high KCl solution with which the contents of the fibers are in equilibrium.

It is something of a mystery that the ability of potassium ions to carry current in an outward direction should depend on the external potassium concentration. Rubidium in low concentration promotes the outward movement of potassium, but there is an optimum rubidium concentration somewhere between 10 and 50 mM. Plainly rubidium does not merely act as if it were sodium or lithium in this situation; a conclusion which is reinforced by the observation that the efflux of potassium is very much reduced by the presence of rubidium, even though the muscle is in a situation where chloride, potassium, and rubidium are in electrochemical equilibrium.

As far as potassium movements are concerned, the classical equations which relate ionic movements to concentration and electrical force are inadequate, except in a purely formal way which turns the permeability constant into an empirical variable. But equally the existing carrier or pore hypotheses are unable to cope with the experimental findings. Better understanding of the mechanisms involved depends upon our ability to separate experimentally the effects of internal and external concentrations on both fluxes at constant potential from the effects on both fluxes of potential at constant concentration.

**References**

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