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Topical Review

Transport of Electrolytes in Muscle

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Summary. The muscle fiber stands alongside the red blood cell and the giant axon as one of the three classical cell types that have had major application in investigating ion transport processes in cell membranes. Of these three cell types, the muscle fiber was the first to provide definite evidence for a sodium pump. The ability of the sodium pump to produce an electrical potential difference across the cell membrane was also first demonstrated in muscle fibers. This important property of the sodium pump is now known to have physiological significance in many other types of cells.

In this review, electrolyte transport investigations in skeletal muscle are traced from their inception to the current state of the field. Applications of major research techniques are discussed and key results are summarized. An overview of electrolyte transport in muscle, this article emphasizes relationships between the muscle fiber membrane potential and ionic transport processes.

Key words ion transport · muscle membrane · Na:K pump · ion fluxes · electrolytes · conductance

Distribution of Electrolytes in Muscle

Potassium ion is the major internal cation of muscle fibers, contributing approximately one-half of the internal osmotic pressure. As the plasma K concentration is only a few millimolar in vertebrates, the ratio $[K^+]_i/[K^+]_o$ lies between 25 and 50 for most muscle fibers. The possibility that the K ion distribution in muscle fibers could be explained as a Donnan Equilibrium had early consideration in view of the known presence of internal nondiffusible anions and the apparent ease of KCl entry into fibers [22]. When Ling and Gerard [101] first applied the microelectrode method to measurements of the membrane potential of frog muscle fibers, quantitative predictions could be further tested. When the value of $[K]_o$ was about 1 mM, the measured value for the membrane potential, E_m, was -84.5 ± 6.0 mV. The equilibrium potential for K ions is given by:

$$E_K = \frac{RT}{F} \ln \frac{[K]_o}{[K]_i} \qquad (1)$$

where, R, T, and F have their usual significance.

These early measured values of E_m were considerably lower than the predictions of Eq. (1). The low potentials and low values for the slope of E_m plotted against log $[K]_o$ obtained by Ling and Gerard [102] and Jenerick and Gerard [82] were later attributed to allowance of insufficient time to reach new equilibria and to junction potentials at the microelectrode tip [32, 115]. The use of microelectrodes filled with 3 M KCl to reduce electrical resistance and junction potentials was introduced by Nastuk and Hodgkin [115] who measured an E_m of -88 mV for frog sartorius fibers with 2.5 mM K^+ in the Ringer's solution. The need to select microelectrodes with low "tip" potentials was emphasized by Adrian [1] who measured a mean value of -92 mV for the membrane potential of frog sartorius fibers in Ringer's solution with $[K]_o = 2.5$ mM. At high values of $[K]_o$, the slope of E_m plotted against log $[K]_o$ is 58 mV for a tenfold change in $[K]_o$ [1, 32, 73]. At plasma levels of K, however, the value of E_m is considerably less negative than E_K which has a value of -101 mV when $[K]_o = 2.5$ mM compared to an E_m of -92 mV.

For precise calculations, Eq. (1) should contain ion activities rather than ion concentrations. Until the advent of ion selective electrodes, ionic activities inside the cell were unknown. The fact that a slope of 58 mV is obtained at higher K concentrations when E_m is plotted versus log $[K]_o$ has been used as an argument that activity coefficients, at least for K ions, must be about the same inside and outside of the fibers. The possibility that the significant departure of E_m from E_K at low $[K]_o$ values is due to differences between inside and outside K ion activity coefficient values has been explored, however [114]. Later direct measurements of the internal K ion activity in frog muscle fibers showed that activity coefficients for K ions are about the same in sarcoplasm as in the external solution [100]. The Na ion and Cl ion activity has also been measured in frog muscle fibers using

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Table 1. Ionic activities and equilibrium potentials in muscle fibers

Ion	Internal		Activity coefficient	Equilibrium potential (mV)
	Concentration (mM)	Activity (mM)		
K	140	108	0.77	−101
Na	10.6	5.5	0.52	61
Cl	3.5	2.8	0.80	−91

Table 2. Membrane potentials in Ringer's solution and plasma

External fluid	$[K]_o$ (mM)	E_m (mV)	E_K (mV)
Frog muscle			
Ringer-Conway	2.5	−92.5	−100.1
Plasma	2.5	−99.2	−100.1
Rat muscle			
Krebs-Ringer	6.5	−77.7	−84.0
Plasma	5.0	−90.1	−91.0

ion-selective electrodes [21, 100]. Values of ionic concentrations, activities, and activity coefficients for K, Na, and Cl are shown in Table 1. Ionic concentrations are those reported in the literature by a variety of investigators [1, 3, 72, 124]. In the case of K ions, the activity was calculated from the activity coefficient measured at a somewhat lower value of $[K]_i$ so that values in the table will correspond to the generally accepted ones for fresh frog muscle [100]. The value cited for $[Na]_i$ was obtained from measurements in which Na washout from fibers in the presence of ouabain is extrapolated to zero time [124]. The difficulties inherent in measurements of $[Na]_i$ in muscle are discussed in this reference. Values calculated from assumed estimates of the extracellular space volume are bound to be inaccurate and too high due to inclusion of Na residing in a rapidly exchanging superficial compartment.

The equilibrium potentials included in Table 1 were calculated using external activities in normal physiological Ringer's solution with the value of $[K]_o = 2.5$ mM, which is near that in frog plasma. Hence, equilibrium potentials can be compared with an E_m value of −92 mV measured in Ringer's solution. The data reported in Table 1 indicate the need for an outwardly directed Na pump and an inwardly directed K pump to maintain a steady state under these conditions. In the case of Cl ions, E_{Cl} and E_m do not differ significantly in most steady-state conditions [21]. In acid solutions, a Cl pump is apparent that will be discussed later.

The data just discussed were obtained on excised muscle in Ringer's solution. There is evidence that some constituents present in plasma but absent in Ringer's solution are important, as different results have been reported when E_m measurements are made with muscle bathed in plasma [88, 91]. Comparisons made between E_m in plasma and E_m in artificial solutions indicate significantly greater internal negativity in the fibers placed in plasma for the same ionic concentrations. Results for both frog and rat muscle are shown in Table 2. The main conclusion from the table is that E_m and E_K do not differ significantly in plasma and that the lower values of E_m in artificial solutions may be a consequence of a departure of muscles from the steady state in such solutions. This matter will be considered further when the Na:K pump in muscle is discussed. The implication of the measurements of E_m in plasma is that a K pump is not necessary to maintain a balanced state for K ions in muscle. However, measurements of K ion fluxes indicate that, under some conditions, a K ion pump does operate in muscle fibers [40, 127].

The fact that E_m is less negative than E_K in fibers placed in Ringer's solution is due to permeability of the fibers to Na. When an inward Na ion passive leakage flux balances an outward leakage flux of K ions, the membrane potential is given by

$$E_m = \frac{RT}{F} \ln \frac{[K]_o + \alpha[Na]_o}{[K]_i + \alpha[Na]_i} \tag{2}$$

where $\alpha = P_{Na}/P_K$. Hodgkin and Horowicz [73] were able to get a good fit of Eq. (2) to their data on E_m measured versus $[K]_o$ over a wide range in concentration with $\alpha = 0.01$. Above a $[K]_o$ value of 15 mM, the preditions of Eqs. (1) and (2) become progressively closer to the same value of the membrane potential. This explains why Boyle and Conway [22] were able to account for KCl entry over a wide range of K·Cl products on the assumption that KCl reaches a Donnan Equilibrium in the fibers. The osmotic behavior of muscle fibers when KCl is added to Ringer's solution is correctly described by the Donnan Equilibrium condition $[K]_i[Cl]_i = [K]_o[Cl]_o$ as long as the value of $[K]_o$ is sufficiently high. The original simplified osmotic theory for muscle fibers has been suitably modified to take account of permeability to Na ions [2, 71]. The steady-state condition now becomes: $([K]_i + \alpha[Na]_i)[Cl]_i = ([K]_o + \alpha[Na]_o)[Cl]_o$. The new relations obtained for fiber volume have been subjected to ample experimental test with satisfactory results [2].

The Na, K, and Cl ion distribution in skeletal muscle fibers can be summarized by stating that K and Cl ions are close to an electrochemical equilibrium, whereas a strong Na pump is required to maintain a steady state for Na ions. Under some condi-

tions, a K pump is required to maintain a steady state for K ions. The determining factor for K pump action is the electrical activity of the Na pump which is discussed in a later section.

Measurement of Ion Fluxes with Isotopic Tracers

The use of radioactive isotopes to trace ion movements in muscle fibers was introduced early in the evolution of electrolyte transport investigation [27, 37, 59, 63, 93]. The criteria for unidirectional transmembrane flux measurement by the tracer method are mainly that the isotope be well mixed in the fiber interior and that the rate constant directly applicable to the membrane process involved be unambiguously extractable from the data. In the case of whole muscle tissue containing numerous fibers, the slowing effect of extracellular or interfiber diffusion must also be taken into account [63, 93]. Flux measurements are technically easier if the fiber or tissue is in a steady state for the ion traced. This is not an absolute requirement, however, as long as it can be shown that the measured influx and efflux are quantitatively consistent with the net ion concentration changes analytically measured [126].

The criteria for unidirectional flux measurement have been satisfactorily met in many cases. The applicability of tracer methodology to muscle ion fluxes has not gone unchallenged, however. Inhomogeneity in the distribution of muscle fiber Na has been reported [27]. In muscles exposed to tracer Na and K but not equilibrated with these isotopes to the point of equality of the specific activities inside and outside of the fibers, a nonuniformity of the specific activity within the fibers has been described [65]. If the nonuniformity of specific activity applies to each individual fiber, the criteria of complete mixing of isotope inside of the fiber membrane is not met and accurate unidirectional flux measurement is impossible [60]. The major criticisms of the isotopic method for measuring unidirectional transmembrane fluxes in muscle have come from work on multifibered whole muscle preparations. Deviations from the single-exponential kinetics expected for membrane-limited ion fluxes have been reported both in rat muscle and in frog muscle [27, 39]. Part of the reason for this behavior may be simply due to the dispersion of fiber sizes occurring in whole muscle. Each different fiber size will entail a different rate constant even for a single membrane-limited process. For frog sartorius and rat diaphragm muscle, the dispersion of fiber sizes alone is not sufficient cause for a pronounced biphasic kinetics for K ion movement [27, 39]. For K ion fluxes, however, two major components of flux are present due to both passive and active transport [127, 141]. If one of these flux components changes during the measurement, departures from simple kinetics can be expected. For this reason, it is important to ensure that muscles are in a steady state during flux measurement. When muscles are in or close to a steady state, no significant departure from simple permeability kinetics is observed for frog sartorius muscle. In addition, tests for a uniform internal distribution of ^{42}K ions indicate the presence of a superficial rapidly equilibrating compartment that is not large enough to seriously interfere with the measurement of transmembrane K fluxes [126]. The question, therefore, is not one of the presence or absence of complicating factors, but is one of degree. For K ions in frog sartorius muscle, whole muscle fluxes show good agreement with those measured on single frog muscle fibers where kinetic anomalies should be absent [72, 126]. In the case of Na ions, a second rapidly exchanging compartment is readily apparent on a semilogarithmic plot of Na washout [93, 124]. Many useful results have been obtained in frog muscle by simply ignoring the initial fast fraction of tracer Na loss [97, 122]. In some cases, the slow intracellular Na pool has been enlarged by enriching the fibers with Na to improve the resolution of active Na efflux components [15, 122].

Individual components of ionic flux will be discussed under separate headings. A few general considerations will be made at this point. One of the tacit assumptions made about cell membranes is that their conductance measured electrically is due to current carried by ions across the membrane. One would, therefore, expect a correlation to exist between electrical conductance and ionic fluxes across the membrane. For an ion in the steady state, the relation between conductance and ion flux is:

$$G=\frac{F^2}{RT}j \tag{3}$$

where G is the conductance per unit area and j is ion flux. Both conductance and ion fluxes are known with sufficient accuracy in frog muscle to enable a test of Eq. (3) to be made. About two-thirds of the membrane conductance of frog muscle fibers is due to Cl ions and almost all of the remainder is due to K ions [73]. The comparison between measured values of partial ionic conductance and values calculated from ion flux data using Eq. (3) is made in Table 3. The measured values of conductance are average values of the two most realiable sources [73, 130]. The flux values cited are from the literature and, for K ions, are consistent with both whole muscle and single fiber data [3, 72, 126]. The electrical conductance for $[K]_o=5$ mM was not available and, as K flux is linear with $[K]_o$ in this range, the K conduc-

Table 3. Ionic membrane conductances compared with values calculated from flux theory

Ion	$[K]_o$ (mM)	Flux (pmol/cm^{2}sec)	G_{meas} (μmho/cm^2)	$G_{Eq^-(3)}$ (μmho/cm^2)
Cl	2.5	10	187	38
K	2.5	6.5	74	26
K	5.0	12.5	148	48

tance was also assumed to be linear. It is at once apparent from Table 3 that the conductances calculated from fluxes are too low, by large factors, to agree with the membrane conductances electrically measured. The combined fluxes of K and Cl ions account, via Eq. (3), for only about 25% of the total measured membrane conductance. The reasons for the disagreement have not yet been made clear.

As Cl ions contribute most to membrane conductance, disagreement is most serious for these ions. Part of the problem may lie in the difficulty of measuring Cl flux accurately [3]. Chloride ions are present at a low concentration inside of the fibers and most muscle Cl is, therefore, extracellular. Influx measurements for Cl ions are precluded and use must be made of efflux data. The rate constant for loss of muscle Cl is very high and difficult to distinguish from extracellular space washout. It is possible that the true Cl ion flux is several times higher than the value cited and, hence, is impossible to measure accurately. This cannot be the problem with K ions, however. Here the disagreement is much larger than could be accounted for by any corrections in the measured flux values. In this case, it must be assumed that the premise of independent ion fluxes upon which Eq. (3) is based is not valid and that there exists a mutual interference of the unidirectional K fluxes with one another [75]. For these conditions, the right-hand side of Eq. (3) must be multiplied by a factor, n, which represents the number of membrane positions at which interference occurs during passage of a K ion across the membrane. The data in Table 3 indicate that such a factor would have to be about 3 to resolve the disagreement. More will be said about nonindependent K fluxes in the next section where models for K ion transport in muscle are considered in more detail.

Mechanisms in Muscle Potassium Transport

Active K transport will be discussed as part of the Na:K pump. Passive K ion movements have been studied in muscle both by electrical and isotopic flux methods. Boyle and Conway [22] observed that KCl enters muscle fibers with ease. It is not surprising, then, that electrical conductance measurements also reveal a rapid K entry into fibers. Katz [84] found that the K conductance for fibers in an isotonic K_2SO_4 solution was much higher for inward K ion movement than for outward movement and termed the behavior *anomalous rectification* as it is opposite to that expected for K channels during an action potential. Other studies show that muscle fiber permeability to K ions remains high for maintained inward currents, but falls to low values for outward currents in contrast to the permeability to Cl ions, which remains constant and independent of the direction of ion movement [2, 73].

When K ion fluxes are measured using ^{42}K, K influx rises as $[K]_o$ is increased. The increase in K influx is linear with $[K]_o$ until concentrations are reached which depolarize the membrane considerably. The influx of K ions declines below the linear relation in a manner that can be accounted for by a drop in the driving force for inward K movement as defined by the constant field flux equation [74, 118]. Under conditions of zero total membrane current and when the K fluxes are approximately in balance, the permeability coefficient for K ions, P_K, is independent of both $[K]_o$ and E_m, and is about the same for K efflux as for K influx [118]. As this behavior differs markedly from that observed when P_K is deduced from K conductance measurements made electrically, possible explanations must be considered. One possibility, previously mentioned, is that K ion movement occurs in channels in which influx and efflux of K ions interact due to their inability to pass one another in the channel [73, 75]. In this case, permeability measurements made by the isotopic flux method would be anomalous and not the electrically measured conductance, since not all the ^{42}K ions that entered a channel would pass through to the other side of the membrane. Another possibility is that the fraction of open K channels is determined by the degree of usage by moving K ions. According to this hypothesis, a net stream of K ions would be able to maintain a channel in an open state. It could be postulated that the opening stream of K ions is provided much more easily by entering ions than by ions leaving the fiber, possibly because of the unfavorable electrical field inside the channel in the case of exiting ions.

A useful means to test ion flux data for independence of the unidirectional fluxes is the flux ratio equation of Ussing [134]:

$$\frac{j_o}{j_i} = \frac{[C]_i}{[C]_o} e^{\frac{ZE_mF}{RT}} \tag{4}$$

where j_o refers to efflux, j_i to influx, C to ionic concentration, and Z to ionic valence. This equation is obeyed if ion fluxes occur passively and independently. If ion unidirectional fluxes are not independent, for example, if passing is not permitted in an

Table 4. The flux ratio for K ions in frog muscle fibers

$[K]_o$ (mM)	$[K]_i$ (mM)	E_m (mV)	E_K (mV)	$(j_o/j_i)_{meas}$	$(j_o/j_i)^n_{Eq.\ (4)}$	n
0.2	140	−113	−165	9.2	7.5	1
2.5	140	−92	−101	1.20	1.45	1
5.0	140	−80	−84	1.02	1.17	1
50	230	−18	−38	5.0	5.0	2

ionic channel, then the right-hand side of Eq. (4) must be raised to a power representing the number of sites within the channel at which interaction can occur [75]. Ample tracer K flux data is available to permit a test of Eq. (4) in frog muscle [76, 120, 126]. The data are summarized in Table 4 where measured values of the ratio of K efflux to K influx are compared with values calculated from Eq. (4) with the power factor, n, chosen as the nearest integer for approximate agreement with the data. Much of the data is in agreement with Eq. (4) predictions.

However, when K ions are close to a steady state, the flux ratio approaches 1 and even large values of n would not be detected since $1^n=1$. When $[K]_o$ is reduced over 10-fold from plasma values, there is a large driving force for outward K ion movement and there still exists fairly good agreement between data and Eq. (4) when the flux ratio is about 9. A marked departure of the measured K flux ratio from the values predicted by Eq. (4) is not observed until fiber membranes are highly depolarized ($E_m=-18$ mV) and the driving force is large and outward [76]. In this case, the exponent required for agreement of the measured flux ratio with theory is $n=2$. Under these conditions, the K fluxes are clearly nonindependent. It is clear that the reason is not entirely due to the driving force as, for $[K]_o=0.2$ mM, the driving force is outward and even larger. To produce the fibers with potentials of −18 mV, equilibration in high concentrations of KCl was required prior to flux measurement [76]. Under these conditions, P_K falls to very low values and the membrane potential is determined by the chloride ion ratio [73]. Such chloride-clamped fibers are useful for measuring K fluxes at varying values of $[K]_o$ but constant values of E_m.

An alternate way to look at the K fluxes in muscle fibers is to regard them as produced by reactions of K ions with a membrane-located carrier [76, 119]. A plot of K influx versus $[K]_o$ at varying values of E_m appropriate for each value of $[K]_o$ can be fit by Michaelis-Menten kinetics with a k_m value of about 40 mM (solid dots, Fig. 1). Values of K efflux at a constant E_m value of −18 mV fit the same curve (open triangles). Values of efflux were normalized in this case as different frog muscle fibers were used [76].

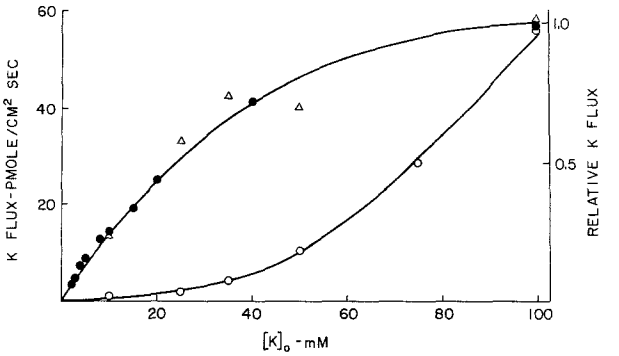

Fig. 1. Potassium fluxes in frog skeletal muscle plotted against $[K]_o$. Fluxes were measured using ^{42}K ions. Symbols are as follows: (●) K influx in frog sartorius muscle at variable E_m, data from Sjodin [118]. (△) K efflux and (○) K influx in frog semitendinosus muscle with E_m held constant at −18 mV, data from Horowicz et al. [76]

Normalized values of K influx with E_m held constant at −18 mV are plotted as open circles in Fig. 1. It is clear that K influx remains very low when E_m is held constant at −18 mV until high values of $[K]_o$ are reached. Under these conditions, the data can be fit by assuming that K ions react with a membrane site according to a second order reaction [76]. All of the isotopic K flux data discussed can be rationalized by supposing that K fluxes in muscle fibers are determined by reactions of K ions with an immobile membrane control site according to simple Michaelis-Menten kinetics and with a mobile carrier site with second-order kinetics. The kinetic nature of the curves in Fig. 1 suggest that a large internal electrical negativity, such as that existing in normal fibers, greatly facilitates carrier loading from the outside solution. Under these conditions, reaction of K ions with the control site is rate limiting and Michaelis-Menten kinetics are obeyed, even at low values of $[K]_o$. In depolarized fibers, however, the second-order carrier reaction is rate limiting and second-order kinetics are followed rather than Michaelis-Menten kinetics. It is interesting that, when second-order kinetic reactions are rate limiting, the flux ratio equation must be squared for agreement with data whereas Eq. (4) is obeyed when both K efflux and influx follow Michaelis-Menten kinetics. The model of Horowicz et al. [76] helps to reconcile ionic carrier phenomena with electrochemical considerations such as the ionic flux ratio.

Permeability to Foreign Cations and Interactions with K-like Cations

It has already been pointed out that muscle fiber membrane discriminates markedly between K and Na ions, the ratio of P_{Na}/P_K being about 0.01. Relative permeability to other ions can be determined from

their effects on the membrane potential. Lithium ions, for example, do not change the membrane potential significantly from that observed in Na-Ringer's solution and one concludes that $P_{Li}=P_{Na}$ [98]. Thallium ions, on the other hand, depolarize frog muscle fiber membranes to the same extent as do K ions, a slope of 58 mV being obtained when E_m is plotted against the logarithm of ion concentration [113]. It is clear that P_{Tl} and P_K must have about the same value. The ionic crystal radii of Na and Li are small: 0.95 Å (Na^+), 0.60 Å (Li^+); while the crystal radii of K and Tl are larger: 1.33 Å (K), 1.44 Å (Tl). As $P_{Li}=P_{Na}$ and $P_{Tl}=P_K$, ionic crystal radius may play a significant role in determining ion selectivity in muscle fiber membrane, possibly by determining the fit of the ions to specific pores or channels [108, 109]. Due to the dependence of the electrical field at the ion surface on the crystal radius, hydration energy also shows a strong dependence on the ionic radius. The mutual interaction energy of an ion with a fixed anionic site in the membrane depends on ionic radius as well. A theory of ion selectivity for excitable membranes based upon dehydration of the ion and association with a fixed anionic membrane site has been proposed [47].

The permeability of muscle membrane to other alkali metal cations has been determined both by flux measurements and by membrane potential measurements [13, 108, 118]. The observed rank order of permeability coefficients is $P_K > P_{Rb} > P_{Cs}$. It is noteworthy that the permeability to Cs ions with a crystal radius of 1.69 Å is very low. It is evident that the cations that penetrate the muscle fiber membrane with the greatest ease are those of intermediate size; the permeability to cations of either low or high crystal radius is low.

Available evidence favors the view that K, Rb, and Cs ions use the same channels or membrane sites to cross the membrane as these ions compete with one another to enter the fibers [20, 119]. The presence of either Rb or Cs ions in the external medium interferes with the efflux of K ions from muscle fibers and reduces the value of P_K [10, 118]. When Rb ions are present inside the muscle fibers but are absent from the external solution, however, there is no slowing effect on the K efflux [121]. Small amounts of Rb inside the fibers, on the other hand, slow the movement of K ions into the muscle even in the absence of external Rb [20, 121]. No complete explanation is yet available for all of these effects. The effect of external Rb and Cs ions on K influx can be accounted for kinetically on the basis that they compete with external K ions for occupancy of externally-directed membrane sites [119]. No doubt the two-site model previously considered could be suitably modified to account for at least some of the other effects [76]. It could be postulated, for example, that Rb and Cs ions compete with K ions outside of the membrane for occupancy of the control site conferring Michaelis-Menten kinetics on the K fluxes. Both K influx and efflux would become inhibited according to this mechanism. Additional modifications would be required to explain the other effects, e.g., the ability of internal foreign cations to block K influx but not K efflux.

Alternatively, it is possible that the cations cross the membrane by passing through specific channels. It could be postulated that Rb and Cs ions can enter the K channel but can pass through the channel only at a low rate. A difficulty with this model is that Cs ions cross the membrane at a much lower rate than do Rb ions, yet Cs is no more effective a K blocker than is Rb [20, 119]. Whatever the mechanism for cation penetration, it is clear that the rate of membrane crossing is only one factor in flux interaction. The ions must use or have access to the same sites or channels. Sodium ions, for example, cross the membrane as slowly as Cs ions yet they are without effect on the K fluxes [64]. Clearly, Na ions use different channels. Also, anions, which must use different channels or membrane sites, are without effect on the K fluxes in muscle [46].

The influence of foreign cations on the membrane conductance of frog muscle fibers has also been investigated [4, 130]. External Rb ions interfere with the anomalous rectification of the muscle fiber membrane due to K ion inward current. In addition, Rb ions themselves are unable to carry appreciable current through the inward rectifying pathway [4]. At large external Rb concentrations, the I–V relation for fibers is approximately linear over a large range of E_m. On the other hand, for fibers with most of the internal K replaced by Rb ions, anomalous rectification is almost normal when $[K]_o=100$ mM. The finding that external Rb interferes with inward K ion currents agrees with isotopic K influx studies. The finding that internal Rb ions do not interfere with inward K current, however, is at odds with the tracer K measurements where internal Rb does reduce ^{42}K influx. The conductance measurements were apparently only made at very high values of $[K]_o$, however, where the inhibition due to Rb may have been overcome. This interesting area should be explored further to seek consistency between ion flux and membrane conductance measurements. At present, one can say that at least some of the pathways for K ion movement revealed by electrical conductance measurements can also be demonstrated by isotopic flux measurements.

Barium ions present externally are particularly

powerful blockers of both K conductance and ^{42}K flux in frog muscle fibers [69, 127, 130]. Almost all of the passive K flux measured with ^{42}K ions can be blocked with mM concentrations of external Ba ions [127]. Similar concentrations of Ba also block a fraction of the membrane conductance quantitatively about equal to the entire K conductance [73, 130]. The fact that Ba ions block both K conductance and ^{42}K fluxes so effectively supports the idea that isotopic K fluxes and electrically-measured K currents reflect the same membrane process. It is noteworthy that a blocking action of Ba ions on K channels or sites was predicted much earlier on the basis that the crystal radii of K and Ba ions are about equal [108].

The Na Pump in Muscle Fibers

Skeletal muscle was the first tissue for which accurate quantitative data on the distribution of electrolytes made it necessary to postulate the existence of a Na pump. The term sodium pump was apparently first used by Dean [41] who also termed metabolically-linked ion movements "active" to distinguish them from those due to diffusion which he called "passive". Active transport of Na was first experimentally demonstrated by Steinbach [131] who showed that frog muscles enriched with Na by immersion in a K-free medium extruded the gained Na against an electrochemical gradient if K ions were readmitted to the medium. These experiments have received ample confirmation and have also been extended to mammalian muscle [34, 42, 53].

Muscle fibers that have gained Na and lost K, lose the ability to extrude Na ions and recover K ions if various cardio-active steroids such as ouabain or strophanthidin are present in the external medium [83, 104]. Since these early studies, ouabain and strophanthidin have been widely utilized as Na pump inhibitors in a wide variety of cells and tissues. Most of the Na efflux in muscle fibers can be abolished by ouabain or strophanthidin and Na-free solutions [23, 77, 123]. In frog sartorius muscle, the fraction of Na efflux abolished by Na-free solutions increases as $[Na]_i$ is made low [123]. The fraction of Na efflux in muscle sensitive to external Na has been termed "exchange diffusion"; this will be discussed in a separate section [97, 135].

The involvement of K ions with the Na pump in muscle fibers was discovered very early. Muscles of K-deprived rats take up Na ions instead of K ions [70]. Potassium ions in the medium are required for net Na extrusion [131]. Removal of K ions from the external medium results in a slowing of tracer Na loss from muscle fibers [45, 93]. The action of strophanthidin and external K deprivation on Na efflux from fibers is about the same, supporting the view that external K ions activate the Na pump and that cardioactive steroids inhibit the K-activated fraction of the Na pump [45]. Measurements of the action of strophanthidin or ouabain on Na efflux from muscle fibers bathed in K-free media indicate that an inhibition of Na transport still persists [12, 122]. This suggests that Na ions present in the external medium can also activate the Na pump. The influx of Na is normally insensitive to Na pump inhibitors, but becomes sensitive to ouabain or strophanthidin in a K-free solution [77, 96]. It appears that a component of the Na influx is coupled to Na extrusion by the pump under these conditions. When both K and Na ions are removed from the external medium, a small sensitivity of the remaining Na efflux to ouabain or strophanthidin is still present [12, 122]. This pump activation could be due to K ions leaking out of the fibers and accumulating in a region close to externally-directed pump sites.

The kinetics of the activation of the Na pump in frog muscle fibers by external K and Na have been investigated [122]. In K-free solutions, external Na ions activate the Na pump according to Michaelis-Menten kinetics with a K_m of about 30 mM. When the Na pump is activated by K ions, the kinetics depend on the external Na ion concentration. In the absence of Na in the bathing medium, activation by external K ions follows Michaelis-Menten kinetics with a K_m of 3.3 mM for whole sartorius muscle and 0.3–0.4 mM for single fibers or small bundles of fibers from the frog semitendinosus muscle [105, 122]. The reason for the large discrepancy is unknown. The difference could be due to the use of different fiber types or it could be due to the difficulty in achieving truly K-free conditions outside of the fibers in whole muscle measurements. In this case, one might be adding increments in $[K]_o$ to an already significant value rather than to an initial value of zero. That this is probably the actual reason is suggested by the fact that the lower value of K_m agrees well with that observed in other cell types, e.g., red blood cells. When Na is added to the medium, inhibition of the pump results, the degree of inhibition growing as $[Na]_o$ is elevated. These kinetic results are summarized in Fig. 2. The data fit a theory in which it is supposed that three membrane subsites are involved in Na pump activation by external K ions [122]. External K and Na are assumed to compete for site occupancy, and the presence of a Na ion on any one of the three subsites suffices to block activation by K at that group of sites.

The role of $[Na]_o$ in modulating the pump rate under physiological conditions is unknown. In the

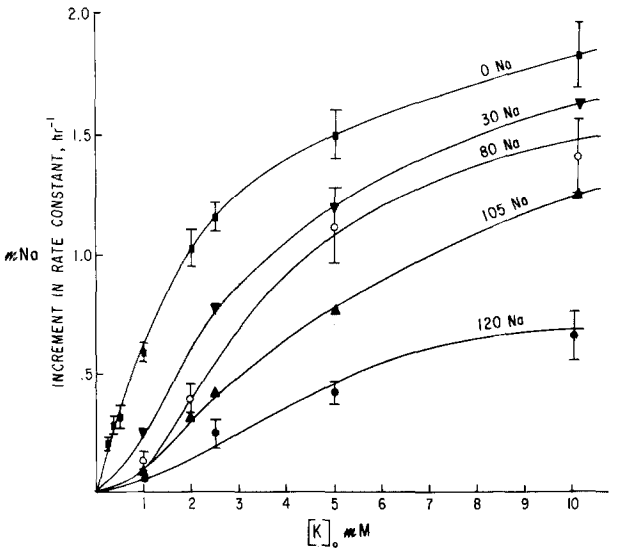

Fig. 2. The K-sensitive rate constant for pumped Na efflux in frog sartorius muscle is plotted against $[K]_o$ for different external Na ion concentrations (mM concentrations above each curve). Tris was used to balance the osmolarity to that of 120 mM NaCl. Bars refer to ±1 SE for 6 to 9 measurements. The figure is taken from Sjodin [122]

physiological range of values for $[K]_o$ and $[Na]_o$, the fractional change in pump rate is significant when either concentration is changed. A regulatory role for $[Na]_o$ is entirely possible. The significance of such regulation remains unclear, however. The inward leakage rate for Na ions in fibers decreases linearly as $[Na]_o$ is reduced [124]. The pump thus apparently extrudes Na faster when the need to do so is diminished. In addition to effects of $[Na]_o$ at externally-directed pump-activation sites, the electrochemical gradient for Na ions is altered when $[Na]_o$ is changed. This is the basis of the "critical energy barrier" theory for Na extrusion in muscle fibers invoked by Conway [33]. According to this theory, net extrusion of Na from fibers only occurs if the electrochemical gradient for Na ions is reduced below a certain threshold value [35]. Thus, raising the value of $[K]_o$ alone would be insufficient to induce net Na extrusion from fibers and it would be necessary to also reduce the value of $[Na]_o$. This theory has been challenged and evidence provided that it is not necessary to reduce the value of $[Na]_o$ to induce Na extrusion from Na-enriched frog sartorius muscle [132]. It now appears that there do exist threshold concentrations for K and Na ions that have to exist in order for the Na pump rate to exceed the inward Na ion leakage rate and so permit Na extrusion to occur [122]. The kinetic basis for the threshold $[K]_o$ and $[Na]_o$ values is apparent in the data illustrated by Fig. 2. According to this view, the "critical energy barrier" is only an apparent one and results merely from the fact that the pump rate is too low to balance or exceed the Na inward leakage flux under some conditions. At low values of $[K]_o$ where net Na extrusion does not occur in Ringer's solution, net extrusion of Na can be induced by lowering the value of $[Na]_o$ [122]. If $[K]_o$ is high enough, say 10 mM or above, it is not necessary to lower the value of $[Na]_o$ for net Na extrusion to occur [122, 132].

The Na pump rate is also sensitive to the internal Na ion concentration [97, 112]. As the work of the Na pump is to maintain the value of $[Na]_i$ at normal values, one would expect it to operate at a faster rate at higher values of $[Na]_i$. It is not surprising, then, to find that the Na pump is activated by internal Na ions. As $[Na]_i$ falls to low values, the rate of pump operation declines sharply. As the value of $[Na]_i$ increases in the range 0 to 8 mM, activation of the pump follows kinetics that suggest that three Na ions must occupy internally-directed membrane sites for pump activation to occur. In this concentration range, the Na pumping rate is proportional to $[Na]_i^3$ [97, 112]. Above a concentration of about 8 mM, the Na pumping rate increases linearly with further increase in $[Na]_i$ and finally begins to saturate at high values of $[Na]_i$. From $[Na]_i = 8$ to 40 mM, Na pump rate varies linearly with $[Na]_i$ [122].

The internally and externally-directed membrane sites at which Na and K ions activate the Na pump vary in their selectivity to other cations. The internally-directed sites are very specific for Na ions. Lithium ions, which use Na permeability channels about as effectively as do Na ions themselves, are able to activate the Na pump to only a very limited degree at internal sites [98]. On the other hand, Li ions are effective in activating Na extrusion at the externally-directed sites at which K ions normally activate the pump [9, 12]. Other cations which have a K-like action when present in the external medium are Rb^+, Cs^+, Tl^+ and NH_4^+ [5, 11, 13, 113]. The order of selectivity is similar to that observed with the Na^+-K^+-ATPase activity in isolated membrane fragments [68, 128].

As the Na-pump must extrude Na ions against both a concentration gradient and an electrical potential gradient, a possibility exists that the pump is sensitive to the membrane potential as well as to ionic concentrations. A possible involvement of the electrochemical potential gradient for Na ions in limiting the active transport of Na has already been considered. If an energy barrier is an important factor, depolarizing the fiber membrane should be as effective as lowering the value of $[Na]_o$ in activating the pump. A role of membrane depolarization in pump activation has been postulated [77, 78]. The evidence was based mainly on similar actions of K and azide on

both the membrane potential and on the strophanthidin-inhibitable Na efflux. The actions of azide and K ions on Na efflux have been shown to have quite different properties, however [15]. Also, when sartorius fibers are depolarized to −23 mV, the Na pump is not activated but is slightly inhibited in a K-free solution [15, 16]. It could be argued that both membrane depolarization and the presence of external K ions act together to activate the pump. However, when $[K]_o$ is varied for fibers that are chloride clamped to a constant potential of −23 mV, the kinetics of pump activation closely match those observed when E_m varies. It is clear that membrane depolarization *per se* does not activate the pump and that changes in membrane potential do not alter the way in which K ions activate the pump. Again, evidence favors the view that the electrochemical gradient for Na ions itself does not influence the pump rate significantly but that ionic concentrations themselves do.

The Na Pump in Muscle Fibers is Electrogenic

One of the as yet unresolved paradoxes of Na ion transport in muscle is the fact that, though the Na pump is apparently uninfluenced by E_m over a wide range, the pump itself produces a hyperpolarization that contributes to the membrane potential [5, 54, 89, 110]. This electrical action of the Na pump has been termed "electrogenic." The main properties of the membrane hyperpolarization produced by the pump are that its magnitude correlates well with the amount of pumped Na efflux, it is very sensitive to temperature, and it is abolished by ouabain or strophanthidin. The Na pump can hyperpolarize the fiber membrane to values more negative than E_K. At very high Na extrusion rates, the amount of hyperpolarization can be as high as 40 mV [54]. The electrogenic action of the Na pump can best be demonstrated in Na-enriched muscles where net Na extrusion rates are high. When $[K]_o$ is elevated to 10 mM to activate Na extrusion in such muscles, E_m is more negative than E_K during the period of recovery toward normal Na and K levels inside the fibers. As normal values of $[Na]_i$ and $[K]_i$ are approached, Na extrusion rate declines and E_m approaches E_K in magnitude [5, 89].

The membrane hyperpolarization observed during Na extrusion has an explanation if the Na pump does not operate in an electrically neutral fashion but produces an outward ionic current. This would occur if the pump extrudes more Na ions outward than K ions inward per pump cycle. In this case, the membrane potential for steady-state conditions would be given by the following equation according to Mullins and Noda [114]:

$$E_m = \frac{RT}{F} \ln \frac{\rho P_K[K]_o + P_{Na}[Na]_o}{\rho P_K[K]_i + P_{Na}[Na]_i} \tag{5}$$

where ρ is the pump coupling ratio given by the number of Na ions extruded per K ion pumped inwardly. In the absence of K ion active transport, Eq. (5) reduces to the equilibrium potential for K ions. For an electrically neutral pump, Eq. (5) reduces to Eq. (2), that obtained when all of the inward Na leakage flux is balanced by the outward leakage flux of K ions.

When steady-state conditions do not exist, as is the case during net Na extrusion, Eq. (5) does not hold and the membrane potential is given by:

$$E_m = \frac{RT}{F} \ln \frac{YP_K[K]_o + P_{Na}[Na]_o}{YP_K[K]_i + P_{Na}[Na]_i} \tag{6}$$

where $Y = f_{Na}(1-f_K)/f_K(1-f_{Na})$ and f_{Na} and f_K are ratios of leakage flux to pumped flux for Na and K ions, respectively [127]. Equation (5) for steady-state conditions never predicts a membrane potential more negative than E_K, while Eq. (6) does correctly predict that E_m is more negative than E_K during net Na extrusion. Arithmetically, this occurs in Eq. (6) when Y assumes negative values during net Na extrusion. In the steady state, the value of ρ can be calculated from Eq. (5) when the value of E_m is known along with the ratio P_{Na}/P_K. During net Na extrusion, the value of ρ can be calculated from Eq. (6) if E_m is measured during Na extrusion, if one of the leakage flux/pump flux ratios is known, and if K and Na permeabilities do not change during extrusion or if their changes are known [127].

Active K Ion Transport and Coupling of the Na:K Pump in Muscle Fibers

Having discussed the Na ion part of the pump, the question of cation coupling by the pump reduces to that of determining which cations and how many of them per pump cycle are transported inwardly by the pump. Normally, the cation transported inwardly is K ion. As the Na pump in muscle is electrogenic, it is known that more Na ions are transported outwardly than K ions inwardly. To put pump coupling on a quantitative basis, electrical measurements can be made to determine ρ or active K influx can be measured. Both approaches have been used.

One possibility is that little or no active K transport occurs and that the pump is completely uncoupled. In this event it should be true that $E_m = E_K$. This condition does not hold when E_m is measured in Ringer's solution at normal values of $[K]_o$ but does hold when measurements are made in plasma [88, 91] (*see* Table 2). Attempts to further clarify the cat-

ion coupling of the Na pump in muscle have been made by investigating the ouabain- or strophanthidin-sensitivity of K influx. In normal freshly-dissected frog muscle, K influx is insensitive to these pump inhibitors [94, 123]. For normal values of $[Na]_i$, however, the strophanthidin-sensitivity of E_m is low, amounting to a depolarization of only 2 mV [10]. For the existing value of the membrane conductance to K ions, this change in potential corresponds to a K flux of only 1 pmol/cm^2 sec, which is a small fraction of the total K influx. To this flux, one would have to add any flux due to active K transport by the pump. It is difficult to draw firm conclusions from the K flux data in fresh frog muscle due to problems in resolving strophanthidin-sensitive K influx. It is evident, however, that there is little evidence for active K transport in frog muscle with normal values of $[Na]_i$. When Eq. (4) is applied to K flux data in rat muscle to separate active K influx from passive K influx, about $^2/_3$ of the total K influx is active and the active K influx component amounts to about 3 pmol/cm^2 sec [141]. In another study in rat muscle, both ouabain-sensitive Na efflux and ouabain-sensitive K influx were measured [29]. The ouabain-sensitive Na efflux was 1.45 to 1.67 times the ouabain-sensitive K influx, suggesting a coupling ratio of 1.5 for the Na:K pump. The electrogenic component of K uptake was not determined in this study, however, so that a firm conclusion as to coupling cannot be made from this data.

Further studies of Na:K pump coupling have been conducted using Na-enriched frog muscles. In these muscles, a strophanthidin-sensitive K influx is readily apparent [123]. About one-half the K influx is abolished by strophanthidin or ouabain under these conditions. This correlates well with the increased electrical effect of the Na pump occurring as $[Na]_i$ is elevated. It is possible that this increased K uptake due to pump activity is all due to passive K ion movement in response to membrane hyperpolarization to E_m values more negative than E_K [5]. Thus, Na-enriched muscles reveal pump-related K ion uptake but not necessarily coupled Na:K movements via the pump.

Several lines of evidence, however, indicate the presence of a K ion pump in frog skeletal muscle that is coupled to the Na pump. Under some conditions, net uptake of K ions occurs into Na-enriched muscles that is against an electrochemical gradient [40, 95]. In these cases, the pump fails to generate a membrane potential that is more negative than E_K and only a K pump can produce a gain of K ions by the fibers. Though experiments of this sort demonstrate the existence of a K ion pump in frog muscle fiber membranes, the more normal behavior during net Na extrusion is that the pump makes E_m more negative than E_K. Under these conditions, the extent of K pump operation is uncertain. Further evidence for the existence of a chemically-coupled K pump in muscle fibers comes from studies of foreign cation movement into Na-enriched muscles. Strophanthidin-sensitive uptakes of both Rb and Cs ions occur in Na-enriched sartorius fibers [5, 13]. In these cases, the inward rate of cation movement is too high to be accounted for by passive movement through K channels, due to the low membrane conductance for Rb and Cs ions. It is evident that Rb and Cs ions activate the Na pump and are themselves actively transported inwardly via the K portion of the pump. As Rb and Cs ions produce less of a shunt on the potential generated by the pump than do K ions, the hyperpolarization produced by the pump is higher when activation is by Rb or Cs ions [5, 13]. As the amounts of Rb or Cs ion gained and the amounts of Na ions lost during recovery are about the same, and as most of the Rb and Cs influx is by active transport, the coupling ratio of Na ions extruded to Rb or Cs ions taken up by the pump must be around 1, though it must be somewhat greater than 1 to account for the observed electrogenic effect.

Most of the Rb or Cs entry into Na-enriched muscles is abolished by ouabain or strophanthidin, whereas only about one-half of the K uptake is sensitive to these inhibitors. Also, a large part of the strophanthidin-sensitive K entry is not due to active transport of K ions. For these reasons, it is impossible to investigate the kinetics of the K pump in muscle by the conventional methods. It has been possible to resolve the K pump in frog muscle fibers and to study the kinetics of active K ion transport by using Ba ions to block the K ion conductance [127]. Under these conditions, the lowered membrane permeability to K ions causes fibers to handle K ions in much the same way that they handle Rb or Cs ions. Most of the inward K ion movement is now ouabain-sensitive movement and most is due to active K transport. The behavior of active K transport rate when plotted *versus* $[K]_o$ is shown in Fig. 3. Removal of external Na ions results in an increase in K pump rate much like that observed for the Na pump. External K ions activate the K pump in a Na-free medium according to Michaelis-Menten kinetics with a k_m value of 3.5 mM, which is close to the comparable k_m value for activation of Na extrusion. The rate of active K ion transport has also been measured as a function of $[Na]_i$. The pumped K influx rises linearly with increasing values of $[Na]_i$ (Fig. 4, solid squares). The K pumped flux has the following characteristics: essentially complete inhibition by 10^{-4} M ouabain, activation by $[K]_o$ according to Michaelis-

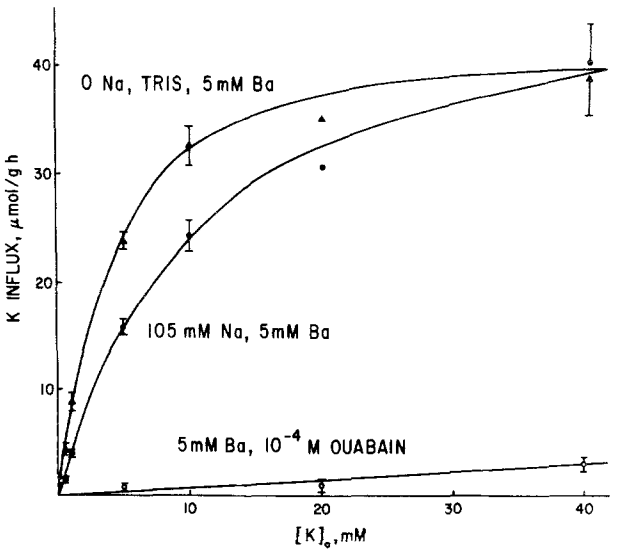

Fig. 3. Potassium influx in frog sartorius muscle in the presence of barium ions is plotted *vs.* $[K]_o$. Muscles were enriched with Na prior to measurement of influx by 24 hr immersion in K-free Ringer's solution at 4 °C. Influx was measured with ^{42}K ions. (▲) K influx in Na-free, Tris-substituted Ringer's solution; (●) Na Ringer's solution; (○) presence of Ba ions and 10^{-4} M ouabain. The figure is taken from Sjodin and Ortiz [127]

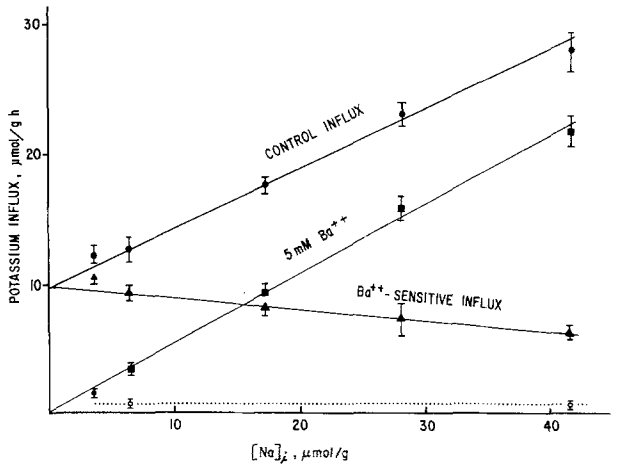

Fig. 4. Components of potassium influx in frog sartorius muscle are plotted *vs.* $[Na]_i$. The value of $[Na]_i$ was set by prior storage in K-free Ringer's solution at 4 °C for varying periods of time. The curves are labeled with the appropriate description except the bottom dotted line, which was obtained in the presence of both 5 mM Ba and 10^{-4} M ouabain. The K influx in 5 mM barium solutions can be taken as the pumped influx of K ions. The figure is taken from Sjodin and Ortiz [127]

Menten kinetics in the absence of $[Na]_o$, inhibition by $[Na]_o$, linear activation by $[Na]_i$ over a wide range, and a rate that approaches zero as $[Na]_i \to 0$. Unlike the pumped Na flux, the K pump does not show a significant residual flux in the presence of ouabain. The remaining K influx after inhibition with ouabain is that due to passive K channels, as is best seen in the presence of Ba ions [127]. The pump-coupled fluxes of Na and K thus can be more-or-less completely inhibited by ouabain or strophanthidin.

Using kinetic data obtained from the ouabain- or strophanthidin-sensitive Na efflux and the ouabain-sensitive K influx measured in the presence of Ba ions, the pump coupling ratio z is constant in the linear region of the fluxes and is equal to 1.5 [122, 127]. In the absence of Ba ions, Eq. (6) has been applied using both membrane potential and flux data to obtain $z = 1.5$ (range = 1.4–1.7). For rat skeletal muscle z is also around 1.5 [29]. Other evidence is available, however, that suggests that z may be variable in muscle fibers [110]. At present, it appears best to regard Na:K pump coupling in muscle fibers as a labile process that can vary according to conditions.

Metabolic Aspects of Na:K Pumping in Muscle

As is the case in red blood cells and squid giant axons, the Na:K pump in skeletal muscle fibers uses ATP as a substrate [43, 111, 136]. When Na-enriched frog muscle fibers extrude Na in the presence of 10 mM external K^+, one molecule of ATP is hydrolyzed for every 2.5 Na ions moved out of the cell. This is shown by poisoning the fibers with dinitrofluorobenzene (DNFB), which inhibits creatine phosphotransferase so that phosphocreatine cannot supply additional ATP. With such muscles, active Na and K ion movements are associated with a decline in ATP levels [43]. When inhibition is with the glycolytic inhibitor iodoacetate, the ATP level also falls during active cation transport even though phosphocreatine breakdown also occurs. In single muscle fibers of the crab, *Maia squinado*, investigation of the Na pump with metabolic inhibitors suggests two energizing systems for the pump, one via Na^+–K^+-ATPase using ATP and phosphoarginine and the other driven by respiration with possible direct coupling of glycolytic energy [19]. It has also been proposed that Na extrusion in frog muscle can occur without any apparent ATP break-down, the energy for extrusion coming from a redox system carrier losing electrons to another system of higher potential [92].

The cation coupling of the Na pump in frog muscle is under metabolic control. When the ratio [ATP]/[ADP] inside the fibers falls, the Na pump shows a Na:Na exchange [85]. Application of the inhibitor DNFB to muscles causes a drop in ATP level and an increase in ADP level. When Na-enriched muscles are treated with DNFB, Na efflux rises for about 30 min and then declines. The increase in Na efflux

depends upon $[Na]_o$ as it does not occur when external Na is replaced with Li. The increase in Na efflux observed with DNFB thus represents a Na:Na exchange via the Na pump and is accompanied by the generation of a strophanthidin-sensitive Na influx. The increment in Na efflux observed with DNFB poisoning also depends upon $[K]_o$ as it is greater in the absence than in the presence of external K ions. That DNFB does not simultaneously act to inhibit the ongoing Na:K exchange via the pump was shown by its failure to significantly alter the strophanthidin-sensitive K influx [86]. Thus, in K-containing media, the Na:Na exchange induced by DNFB is in addition to the Na:K exchange, which operates at the normal rate. The addition of K ions to a K-free medium, however, reduces the strophanthidin-sensitive Na influx induced by DNFB [87]. Thus, as in red blood cells, external K ions inhibit Na:Na exchange by the pump [55]. This explains why DNFB induces less extra Na efflux in the presence of $[K]_o$ than in the absence of external K [85]. The reason for the decline in Na efflux seen after prolonged treatment with DNFB is that ATP depletion begins to limit the rate of pump operation in either the Na:K or Na:Na exchange mode.

Studies have been made with other metabolic inhibitors. In frog muscle, iodoacetamide (IAM) lowers the [ATP]/[ADP] ratio in a manner similar to that observed with DNFB and also affects the Na pump in the same way as does DNFB [85]. In single muscle fibers of the crab *Maia squinado,* the inhibitor atractylate causes a transient stimulation of the Na pump before the onset of pump inhibition [19]. This inhibitor acts by retarding the movements of ATP and ADP across mitochondrial membrane and could lead to changes in ATP and ADP levels similar to those observed with DNFB in frog muscle. The inhibitor azide increases the Na efflux from frog muscle [15, 78]. In Na-enriched fibers, the increased Na efflux is transient and requires the presence of external Na ions. The increased Na efflux observed with azide is abolished by ouabain and is a Na:Na exchange [15]. The action of azide thus resembles that of DNFB and IAM. Azide does act differently, however, with respect to Na:K coupling of the pump. In the presence of azide, K ions activate the Na pump in a normal fashion as shown both by their promotion of net Na extrusion and their effect on ^{22}Na efflux [15]. Potassium ions are not regained during recovery of Na-enriched muscles in azide, however, and ouabain-sensitive K influx falls to low values. The K influx part of the pump functions poorly in the presence of azide, yet activation of Na efflux by K ions is normal. It is apparent that azide uncouples the Na:K pump in muscle unlike the action of DNFB. It is interesting that removal of most of the external Na during inhibition with azide restores much of the ouabain-sensitive K influx. The Na:Na exchange generated by azide thus appears to be at the expense of Na:K exchange yet not at the expense of activation by K ions, which occurs in addition to the activation by Na ions. The uncoupling effect of azide has not been explained, though it clearly shows that cations need not themselves be transported to activate the Na pump. The difference in the actions of DNFB and azide might have a metabolic explanation or an energetic one as azide depolarizes fiber membranes considerably, whereas DNFB has little or no depolarizing action. In the presence of azide, the electrochemical gradient against which K pumping has to occur is increased significantly whereas that for Na ions is decreased. A combination of changes in [ATP]/[ADP] ratio and electrochemical gradients may explain the results with azide.

Action of Hormones on Ion Transport in Muscle

The transport of Na and K in muscle fibers is influenced by insulin, catecholamines, and thyroid hormone.

Net K uptake and Na extrusion in frog muscle is promoted by insulin [90, 129]. In rat skeletal muscle (extensor digitorum longus), insulin causes membrane hyperpolarization, a reduction in both K efflux and influx, and net K uptake [138, 139]. With rat soleus muscle, different results are obtained in that ^{42}K influx is increased by insulin whereas ^{42}K efflux is decreased, but only transiently [31]. In rat diaphragm muscle, tracer ^{24}Na efflux is increased by about 50% by insulin in the absence of external glucose [38]. In frog muscle, tracer Na efflux is also increased by insulin and the increase is blocked by acetylstrophanthidin, which has a ouabain-like action [106]. In rat soleus muscle, insulin increases both the ouabain-sensitive and ouabain-insensitive Na efflux [31].

Many of the effects of insulin just described can be explained as a direct effect on the Na:K pump. Insulin increases the activity of Na^+-K^+-ATPase isolated from both rat and frog muscle [25, 56]. Insulin also hyperpolarizes the membrane of frog muscle fibers [107]. As frog muscle fibers are already known to have a Na pump whose action hyperpolarizes the membrane, almost all of the effects of insulin could be a consequence of a stimulation of the Na:K pump. The mechanism for the Na-pump stimulation by insulin is not known for certain. Moore [106] presents evidence that insulin acts by increasing the affinity of internally-directed pump sites for Na ions. Confirming evidence has been obtained by Kitasato et al.

[99]. Evidence has also been presented that insulin acts to create new active pump sites by unmasking a pool of latent precursor sites [49].

Though a direct action of insulin on the Na:K pump of muscle fibers seems certain, other modes of action cannot be ruled out, particularly in some types of muscle. In rat extensor digitorum longus muscle fibers, the main action of insulin is apparently to lower the P_{Na}/P_K ratio [140]. This cation would lead to membrane hyperpolarization via Eq. (2). In this instance, the hyperpolarizing action of insulin can be ascribed more to permeability changes for Na and K ions than to direct action on the Na pump. Changes in Na and K content would then result from decreased cation leaks at more or less constant pumping rates. Not all rat muscles show this mechanism, however. Diaphragm muscle gives a 50% increase in Na efflux upon adding insulin with no change in P_{Na} [38]. As P_K is slightly reduced by insulin, the effect of insulin in rat diaphragm muscle must be via stimulation of an electrogenic Na pump.

Whatever the details of mechanism and the variations in type of response to insulin, there is general agreement that insulin can hyperpolarize skeletal muscle fiber membrane and can cause additional Na extrusion and K accumulation. Though the biochemical steps are largely unknown, there is evidence that cyclic nucleotides are not involved [49].

Catecholamines also have a hyperpolarizing effect on skeletal muscle membrane. The hyperpolarization is rapid in onset and due to a direct stimulation of the electrogenic Na pump [28]. Both adrenaline and noradrenaline markedly stimulate ouabain-sensitive Na efflux and K influx in skeletal muscle fibers [28, 67]. The effects of catecholamines are mediated by beta-adrenoceptors and probably involve a stimulation of adenylate cyclase. This mechanism differs from that for insulin action on the Na pump, and the effects of insulin and adrenaline on the muscle membrane potential and on muscle Na–K contents should be additive, as observed [52]. Adrenaline accelerates the rate of ^{3}H-ouabain binding but does not influence total specific binding, which indicates that the total number of pump sites is not increased [30].

Thyroid hormone increases the rate of Na and K exchange in rat skeletal muscle [7]. These effects are via an increase in membrane permeability to Na and K ions. Thyroid hormone also stimulates the Na:K pump as shown by an increase in the ouabain-sensitive ^{42}K influx [18]. The pump stimulation has been shown to be a result of an increase in the number of active pump sites as determined by ouabain-binding measurements. The number of K ions transported per ouabain-binding site remains constant, indicating no change in the turnover rate of the pump.

Exchange Diffusion of Sodium

A significant fraction of the Na efflux from skeletal muscle fibers is not inhibited by ouabain or ouabain-like substances. In frog muscle, the ouabain-insensitive fraction of Na efflux is high and amounts to one-half or more of the normal Na efflux [51, 96, 123]. The Na efflux in frog muscle is also highly sensitive to $[Na]_o$ and is reduced by about 50% when Na in Ringer's solution is replaced by Li [97]. The reduction in Na efflux observed in Li media is largely in the ouabain-insensitive component of Na efflux [14, 96].

The hypothesis of a one-for-one Na ion exchange that occurs across the muscle fiber membrane was first proposed by Ussing [135]. According to this hypothesis, a carrier molecule exists in the membrane of muscle fibers that has a high specificity and a high affinity for Na ions. Due to the high affinity, the hypothesized carrier is always saturated with Na and hence shows a one-for-one ion exchange. The hypothesis was useful in reducing the amount of Na efflux in muscle fibers that had to be attributed to active transport in view of the large fraction of the fiber's energy production that would otherwise have to be applied to transporting Na ions. Even if the Na pump were 100% efficient, about 30% of the total energy available would have to be applied to Na extrusion in the absence of any exchange diffusion [135].

With information presently available, there is no longer a need to postulate an exchange diffusion of Na ions on energetic grounds. There are adequate ATP stores and production rate to account for the measured Na extrusion rates [43, 48]. About 16% of the resting oxygen uptake of muscle is sensitive to ouabain and due to the activity of the Na^+-K^+ ATPase. Nevertheless, a Na carrier in muscle fiber membrane that is insensitive to ouabain does exist and is of interest. The original hypothesis has to be modified as the carrier is not of such high Na affinity that it is always saturated. The half-saturation value of $[Na]_o$ for the carrier is 38 mM [97]. Another property of the ouabain-insensitive Na carrier in muscle fiber membrane is that it is blocked by agents which combine with protein SH groups. Ethacrynic acid at 2 mM concentration blocks essentially all of the $[Na]_o$-dependent but strophanthidin-insensitive Na efflux in frog muscle [50]. A similar reduction in Na influx was also seen with ethacrynic acid application, suggesting that the affected carrier is involved in exchange diffusion.

There is some evidence that Na exchange diffusion itself may have an energy requirement in muscle fibers. As originally envisioned, the exchange diffu-

sion process was regarded as a metabolically inert ion exchange. This view now needs modification. Of the remaining oxygen consumption in ouabain-treated muscle fibers, there is a fraction of O_2 uptake that depends on $[Na]_o$ [51]. Total O_2 uptake is reduced by about one-third when external Na is replaced by either choline or Tris in the presence of ouabain. Further evidence that exchange diffusion of Na in muscle is associated with O_2 uptake comes from studies of the effects of foreign tri- and divalent cations on both O_2 uptake and Na efflux [51]. The cations La^{3+}, Mn^{2+}, Co^{2+}, or Ni^{2+} all caused large reductions in the ouabain-insensitive Na efflux. The same cations reduced O_2 uptake in ouabain-treated muscles by about one-third. It is interesting that these foreign cations inhibited O_2 consumption to the same extent as did $[Na]_o$-free conditions. Thus, an important part of the energy metabolism of the fibers seems to be associated with exchange diffusion of Na ions, a process hitherto regarded as metabolically inert. The detailed mechanisms for these interesting effects must await further clarification.

The Na exchange diffusion under discussion at this point must be distinguished from the Na:Na exchange catalyzed by the Na pump. The pump-mediated Na:Na exchange previously discussed is inhibitable by ouabain and sensitive to the presence of external K ions. Neither of these features applies to the process termed exchange diffusion in skeletal muscle. Also, the Na pump and the exchange diffusion carrier respond differently to substitution of Li ions for external Na ions. The Na pump shows an increased activity when external Na is replaced by Li, whereas the Na efflux due to exchange diffusion always decreases in a Li medium [14, 94, 96]. The reason for this behavior is that Li ions activate the pump at externally-directed sites to an extent greater than do Na ions and the exchange-diffusion Na carrier does not show much specificity for Li ions [9, 97].

The view just expounded is that the Na pump and the Na exchange-diffusion carrier represent two distinct processes. Two main facts favor this view: (i) activation kinetics for the Na pump can be effectively studied in Na-free media where, presumably, exchange diffusion is absent, and (ii) Na efflux has other properties that can equally well be detailed in ouabain-treated muscles where the Na^+-K^+-pump and ATPase are inactive.

Other facts dictate the need for caution in arriving at firm conclusions, however. Much interpretation rests on the use of ouabain-sensitivity as a measure of the actively transported Na. Ouabain, or strophanthidin, however, does not completely inhibit the Na pump, even in the squid giant axon, which lacks a significant exchange diffusion of Na [24]. Thus, ATP-dependent Na efflux still persists in the presence of ouabain or ouabain-like substances. The suggestion here for muscle fibers is that exchange diffusion might include some ATP-dependent efflux of Na. The linkage of exchange diffusion to O_2 uptake that has been observed also suggests the exercise of caution in modeling the Na efflux in muscle. A single membrane Na carrier with more complicated properties might be able to explain the observations. However, one must also hold open the possibility that Na pumping and Na exchange diffusion might not only be different processes, but might occur in different fiber compartments [96]. These are clearly areas requiring further investigation.

Anion Movements in Muscle

The main mobile anion in muscle fibers is chloride ion. As previously noted, Cl ions participate with K ions in a Donnan or near-Donnan Equilibrium and contribute about two-thirds of the total membrane conductance [73].

The difficulty in investigating Cl ion fluxes in muscle fibers is that most of the muscle chloride is extracellular, leaving a small fraction of the total Cl in the fiber interior. In whole muscle preparations, this makes it difficult to distinguish a rapidly exchanging fiber compartment from extracellular space washout [3]. When Cl washout from frog muscle is plotted semilogarithmically against the time, both fast and slow components of Cl efflux are apparent [3, 62]. The amount of Cl associated with the slow component is about 15% of the total Cl that would be attributed to the fibers themselves, though slowly exchanging extracellular Cl might account for this fraction [3]. That the slowly moving Cl is associated with a true fiber compartment is suggested by the fact that its properties are different if washout occurs in NO_3-Ringer's solution rather than in normal Cl-Ringer's solution. Also, Cl output is slowed if fibers are first loaded with labeled Cl in a high KCl medium [62]. This suggests that a fiber compartment from which Cl movement is slow has undergone a net gain of Cl ions in the high KCl medium.

Notwithstanding the difficulties in measuring Cl ion flux in muscle, some interesting and useful results have been obtained. Chloride output from fibers is slowed if Cl ions are removed from the medium and replaced with foreign anions [3, 61, 62]. This suggests that some of the Cl efflux from fibers is via a Cl:Cl exchange. It is difficult to measure Cl:Cl exchange in muscle by using Cl-free solutions, however, as the foreign anion used as a Cl replacement itself slows the rate of Cl loss. Anions which slow the rate of Cl output are NO_3^-, I^-, CNS^-, ClO_4^-, Br^-, and

HCO_3^- [3, 61]. If the rate of exchange of fiber Cl with an external anion were the only factor involved, anions which exchange very slowly or not at all with fiber Cl would have the greatest slowing effect on Cl output. Nonpenetrating anions are no more effective than penetrating anions in slowing Cl ion movement [61]. Also, small concentrations of foreign anions slow Cl output more than can be explained by the reduction in $[Cl]_o$. These results suggest that anions compete for membrane sites to cross the membrane. The flux interactions seen with anions in muscle fibers are reminiscent of those previously reviewed for cations. On chemical grounds, two distinct sets of membrane sites seem to be involved in the regulation of cation and anion movements across muscle fiber membrane. It is noteworthy that external anions do not influence the cation movements via the cation-specific sites [46]. External cations can, however, influence the anion movements. The rate of anion loss is greater to a Na medium than to a K medium of equal concentration [61]. This effect might be explained on the basis that, in the K medium, a net inward movement of anion occurs that could impede the rate of anion loss via a single-filing type of mechanism [75].

Some features of the sites used by Cl ions to cross the muscle fiber membrane have been learned through a study of the effects of external pH on Cl ion movement. In frog muscle fibers, the membrane conductance to Cl ions is highly sensitive to pH. The chloride conductance falls in acid solutions and rises in alkaline solutions [79]. One might expect the Cl ion sites in muscle membrane to bear a positive charge. If the site were a charged group on a membrane protein, an acid solution should promote protonation, increase the number of positively charged groups, and increase the chloride ion conductance. This mechanism clearly does not suffice to explain the results with frog muscle. In giant muscle fibers from the barnacle, however, a sharp increase in membrane conductance to Cl ions occurs when the pH is lowered to values below pH 5 [58]. A simple fixed-charge model does, therefore, explain the results in barnacle muscle.

Hutter and Warner [79] have proposed a more complicated model to account for results in frog muscle. A mechanism is visualized in which the affinity of the membrane site for anions increases upon protonation. The permeability to Cl ions is determined by two factors, the fraction of sites occupied by Cl ions and the dissociation rate of the Cl-site complex. Acid solutions increase site Cl affinity but decrease the dissociation rate. The decrease in dissociation rate is greater than the increase in the number of Cl ions in the membrane and, hence, prevails. Thus, Cl ion permeability and conductance both fall in acid solutions [79, 80]. The foreign divalent cations Cu^{2+}, Zn^{2+}, and UO_2^{2+} also decrease the chloride permeability in frog muscle [81]. The same model explains these findings if it is supposed that these divalent cations compete with hydrogen ions for sites that control the permeability to Cl ions. The model is extended by assuming that each divalent cation, when bound to a regulatory site, promotes the binding of one Cl ion at a permeability site. Again, the increased binding of Cl ions is the factor that prevails, and slowed dissociation causes the Cl permeability to fall. Other modifications of the model account for the interactions between monovalent foreign anions and Cl ion movements that were discussed previously. The foreign monovalent anions are assumed to compete with Cl ions for membrane binding sites [81].

Intracellular chloride ion activity measurements (Table 1), as well as membrane potential and osmotic pressure measurements made as the $[K] \times [Cl]$ product is varied, indicate that the muscle chloride is passively distributed, or at least approximately so. A chloride ion pump in muscle fibers would have to operate at a very low rate to be consistent with these observations. A weak chloride pump would be easiest to detect when the Cl ion permeability is made low as in acid solutions. It is interesting that intracellular Cl accumulates to concentration values higher than those expected for electrochemical equilibrium when the external solution is made acid [21, 79]. These observations make it likely that a chloride ion pump of limited capacity is operative in skeletal muscle membrane.

Calcium Movements in Muscle

Calcium ions are of fundamental importance in muscle fibers as they trigger the contraction of the myofibrils. The intense activity of the sarcoplasmic reticulum (*SR*) in sequestering Ca ions via an ATP-dependent process is well known [44, 66]. Following a depolarization of muscle fiber membrane, a transient rise in sarcoplasmic $[Ca^{2+}]$ occurs that precedes a rise in muscle tension [116]. The source of this Ca^{2+} is the terminal cisternae of the *SR*. The released calcium enters the sarcoplasm and is taken up by the *SR* and recycled back to the terminal cisternae [137]. This Ca ion release and resequestration has also been investigated using ^{45}Ca ions in skinned-muscle fiber preparations [133]. These fibers lack a surface membrane, but Ca release can still be induced by elevating the bath Cl ion concentration which depolarizes the internal membranes. The released Ca is effectively reaccumulated by the sarcoplasmic reticulum.

Internal Ca ion movements in skeletal muscle fibers and their role in excitation-contraction coupling have been extensively investigated and recently reviewed [57]. The present purpose is to briefly review Ca ion movements as they relate to the overall economy of the muscle fiber. Skeletal muscle membrane is about as permeable to Ca ions as to Na ions [17]. The concentration of ionized Ca in the fibers is very low, about 10^{-7} M. Given the existing value of P_{Ca} and the magnitude of the electrochemical gradient for Ca ions across muscle membrane, a net stream of Ca ions into muscle fibers must occur. The entering Ca ions must be extruded in some way to prevent overloading of the buffering capacity of the sarcoplasmic reticulum. In addition, extra Ca influx occurs during electrical stimulation of muscle. For frog muscle, the extra influx of Ca amounts to 0.20 pmol/cm^2 per impulse [17]. The additional influx during stimulation is not enough to account for the rise in $[Ca^{2+}]$ that must occur throughout the fiber to initiate a contraction. Nevertheless, any additional net entry of Ca constitutes a load that must eventually be extruded by the surface membrane.

Further evidence that Ca ions that enter the fibers by crossing the surface membrane are not those that activate the contractile elements after an action potential comes from studies in which the value of $[Ca^{2+}]_o$ is buffered with EGTA to below the internal free Ca ion concentration. Under these conditions, Ca influx is of negligible magnitude, yet depolarization still induces contraction [6, 103]. As EGTA has undoubtedly entered the *T*-system in these experiments, it is not likely that a Ca flux across the transverse tubular membrane is the signal for Ca release by the SR.

Mechanisms in the surface membrane for extruding entering Ca ions to prevent excessive accumulation could include both an ATP-dependent Ca pump and a Na/Ca counter transport carrier. An ATP-dependent Ca pump located in muscle fiber membrane would be difficult to demonstrate. The standard method for demonstrating such a pump is that of ATP depletion. Unfortunately, this experimental operation would also affect the rate of the *SR* Ca pumps. A sufficiently specific Ca pump inhibitor does not yet exist. Even if a very specific inhibitor were found, however, probably both *SR* and surface-membrane Ca pumps would be affected.

Evidence exists for a Na/Ca exchanger in single fibers from the giant barnacle, *Balanus nubilus* [8, 117]. Calcium influx is increased when $[Na]_o$ is decreased or $[Na]_i$ increased. Also, Ca efflux drops when Na ions are removed from the external medium. When $[Na]_i$ is elevated, removal of external Na results in a large net gain of Ca ions and contracture. The contracture is accompanied by a large increase in Na efflux [23]. The increase in Na efflux under these conditions requires the presence of external Ca ions [117]. The evidence for Na/Ca counter transport in barnacle muscle fibers is, therefore, quite good.

In vertebrate skeletal muscle, some evidence for Na/Ca exchange also exists, but results are scant and sometimes conflicting. One study in frog sartorius muscle indicates a relationship between total net Ca and Na contents of fibers. Calcium content increased as fibers gained Na in K-free media [36]. At high values of $[Na]_i$, the Na gradient which extrudes Ca ions is lessened and Ca gains would be expected according to a Na/Ca exchange mechanism. In another study in frog sartorius muscle, however, no change in total Ca content was noted over a wide range in values of $[Na]_i$*. Also, no net gain of Ca occurred after storage of muscles in Na-free, Li-substituted media for several hours. On the other hand, an increased Ca influx has been reported in frog sartorius muscle when external Na is reduced using an osmotic equivalent of sucrose [36].

Calcium efflux in frog muscle is sensitive to the external Na concentration. In semitendinosus muscle, the slow component of Ca loss drops by about one-third in the absence of external Na [26]. In frog sartorius muscle, the fast component of ^{45}Ca loss is considerably reduced upon replacing external Na with Li ions. The slow component of ^{45}Ca loss, however, is insensitive to $[Na]_o$ [125].

The details of Na/Ca transport in skeletal muscle have yet to be worked out. Energetic calculations indicate that, with prevailing Na and Ca ion gradients at the normal resting potential of fibers, transport of 3 Na ions inwardly per 1 Ca ion outwardly suffices to account for the inside ionized Ca^{2+} level in frog muscle fibers. If a large fraction of the total Ca influx is due to leakage through membrane channels rather than via the Na/Ca carrier, a larger number of Na ions must be transported per Ca ion. The value of $[Ca^{2+}]_i$ clearly must be set at a resting value such that the *SR* is in a steady state with that value at the total resting Ca level and such that the combined action of Na/Ca transport and an ATP-driven Ca pump in the surface membrane produces a pumped Ca efflux that just balances the inward leakage rate across the surface membrane.

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