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# Sodium and Potassium Permeability of Membrane Vesicles in a Sarcolemma-Enriched Preparation from Canine Ventricle

William P. Schilling, Duane W. Schuil, Ervin E. Bagwell and George E. Lindenmayer
Departments of Pharmacology and Medicine, Medical University of South Carolina, Charleston, South Carolina 29425

**Summary.** Vesicles in a highly enriched sarcolemma preparation from canine ventricle were found to develop membrane potentials in response to outwardly directed potassium and inwardly directed sodium concentration gradients. The magnitude of the potential measured by the fluorescent dye diS-$C_3$-(5) suggested a sodium-to-potassium permeability ratio between 0.2 and 1.0 which is one to two orders of magnitude greater than values obtained for the myocardial cell. Radiotracer techniques were employed to evaluate the permeability coefficients of the isolated cardiac sarcolemma membrane for sodium and potassium under equilibrium conditions (i.e., equal salt concentrations in the intravesicular and extravesicular spaces). The uptake of sodium and potassium was best described by two exponential processes which followed an increment of uptake that occurred prior to the earliest assay time (i.e., 17 sec). The compartment sizes were linear, nonsaturable functions of the cation activity. Evaluation of the rate coefficients of cation uptake by the two exponential processes versus cation activity revealed that sodium influx via the slow process and potassium influx via the fast process varied linearly with cation activity, suggesting that the permeability coefficients were concentration independent for these compartments. Conversely, sodium influx via the fast process exhibited a nonlinear increase with increasing sodium activity, and potassium influx via the slow process appeared to saturate with increasing potassium activity. In general, the permeabilities of the sarcolemma-enriched preparation for sodium and potassium were of equal magnitude. The permeability coefficients were lower than that for the potassium coefficient reported for cardiac cells but are in the range of that reported for sodium.

**Key Words** sarcolemma · heart · potassium permeability · sodium permeability · membrane potential

## Introduction

Under normal conditions, myocardial contraction is triggered by depolarization of the cell which involves activation of fast sodium channels in the sarcolemma (plasma membrane). Activation of these channels requires that the resting (diastolic) potential be greater than about −60 mV (inside negative) (Weidmann, 1955). The primary determinant of steady-state resting membrane potential of the myocardial cell is the relative permeability of the sarcolemma to sodium and potassium.

Measurements of resting membrane potential in cells of intact cardiac tissue have suggested that the ratio of membrane sodium to potassium permeability is between 0.03 and 0.01 (for review *see* Sperelakis, 1979). From radiotracer fluxes in isolated, intact, 19-day-old chick embryo heart, Carmeliet et al. (1976) calculated that the permeability coefficient for sodium ($P_{Na}$) is equal to $4.6 \times 10^{-9}$ cm/sec while the permeability coefficient for potassium is equal to $2.4 \times 10^{-7}$ cm/sec, which yields a $P_{Na}$ $P_K$ ratio equal to 0.019.

Vesicles in a highly enriched sarcolemma preparation from canine ventricle have been found to develop membrane potentials in response to outwardly directed potassium and inwardly directed sodium concentration gradients (Bartschat, Cyr & Lindenmayer, 1980). The magnitude of the potential, however, could be dramatically increased by the addition of the potassium-selective ionophore valinomycin. This suggests that the sodium-to-potassium permeability ratio of the isolated cardiac sarcolemma preparation is much greater than expected from electrophysiological and ion flux studies performed in cardiac tissue. This discrepancy could be accounted for by: 1) a decrease in the permeability of the isolated membrane to potassium, 2) an increase in the permeability of the membrane for sodium or 3) both an increase in sodium and a decrease in potassium permeabilities. The objective of the present study was to evaluate the permeability of the isolated cardiac sarcolemma membrane for sodium and potassium.

## Materials and Methods

### Isolation of the Sarcolemma Preparation

The isolation of sarcolemma-enriched preparations from canine ventricle was accomplished by the method of Van Alstyne et al. (1980). After isolation, the preparation was stored at 4 °C until

use. These preparations consist of membrane vesicles and were osmotically responsive (Van Alstyne et al., 1980).

### Solutions and Reagents

Unless otherwise stated, all solutions contained either 5 mM imidazole-Cl or 10 mM Tris-Cl, pH 7.4. $^{42}K$ (New England Nuclear) was obtained as the KCl salt. $^{22}Na$ (New England Nuclear or Amersham) was obtained as the carrier-free chloride salt. The specific activity of all solutions containing radiotracer was 100 to 1,000 cpm/nanomole cation. The fluorescent dye, 3,3′-dipropyl-2,2′-thiadicarbocyanine (diS-$C_3$-(5)) was a generous gift of Dr. Alan S. Waggoner (Amherst College, Amherst, Mass.). All other chemicals were of reagent grade.

### Activity Coefficients

The concentrations of potassium and sodium were converted in some cases to activities by the following equation:

$$a = f[C] \tag{1}$$

where $a$ is equal to the activity of the cation, $f$ is the activity coefficient and $[C]$ is the concentration of the cation. The activity coefficients for the chloride salts of potassium and sodium at 25 °C (Weast, 1971) were corrected to 5 °C by the following equation:

$$\log f = A\sqrt{\mu} \tag{2}$$

where $A$ is a constant for a given solvent and temperature and $\mu$ is the ionic strength. Values for $A$ at different temperatures were used to calculate the value of $A$ at 5 °C (Maron & Prutton, 1965). It was assumed that the intravesicular and extravesicular activity coefficients were equal.

### Protocol for Measurement of Membrane Potential

Aliquots of the sarcolemma-enriched preparation were allowed to equilibrate at 4 °C for 15 to 18 hr with a solution containing 150 mM KCl, 10 mM counter-cation (i.e., either NaCl, LiCl or choline-Cl) and 10 mM Tris-Cl, pH 7.4, for 37 °C. Aliquots of the loaded preparation (15 μl; approximately 20 μg protein) were placed in a cuvette containing solutions with varying concentrations of KCl. The external potassium plus counter-cation concentration was kept equal to 160 mM. The suspension was allowed to equilibrate at 37 °C in the spectrophotofluorometer for 1 to 2 min at which time 5 μl of 0.3 mM diS-$C_3$-(5) in absolute ethanol was added to the cuvette (final concentration of dye equal to 1 μM) and rapidly mixed with a pipette. The fluorescence change was monitored using excitation and emission wavelengths of 622 and 670 nm, respectively. After approximately 2 to 3 min, 1.5 μl of 100 μM valinomycin in absolute ethanol was added to the cuvette (final concentration of valinomycin equal to 0.1 μM) and the response was recorded.

### General Protocol for Measurement of Radiotracer Associated with Membrane Vesicles

Vesicles in the sarcolemma-enriched preparation were "loaded" internally with different concentrations of salt by allowing the freshly isolated preparation to equilibrate with a solution containing KCl or NaCl for 15 to 18 hr at 4 °C.

Unless otherwise stated, all reactions were carried out at 5 °C. Aliquots of these loaded suspensions were added to reaction media containing the desired concentrations of KCl or NaCl, 5 to 10 mM buffer, pH 7.4, and tracer amounts of $^{42}K$ or $^{22}Na$. Subsequently, duplicate samples (taken 4 sec apart) were filtered at varying times (Millipore, 0.45 μ; Hoeffer filtration apparatus), washed three times with 3-ml aliquots of ice-cold solution containing 200 mM KCl or NaCl (i.e., the cation in the loading medium), and 5 to 10 mM buffer, pH 7.4. The filters were then removed from the filtration apparatus and placed in scintillation vials; the radioactivity trapped on the filter was measured using gamma emission spectroscopy (Beckman G8000 Gamma Counter). The data were corrected for decay of $^{42}K$ that occurred during the counting procedure.

The amount of protein per filter was approximately 15 μg (*see* Results) and the average cpm per filter after the wash procedure was always greater than 1,000 cpm. Filter blanks were between 250 and 500 cpm depending on the number of counts added to the reaction medium. These values were determined by addition of the reaction medium (without the membrane preparation) to the filter. This was followed by the standard washing procedure.

### Data Analysis

The radiotracer flux data were analyzed via a nonlinear least-squares, iterative technique (Nelder & Mead, 1965; Lam, 1970) with respect to a number of mathematical models. The data were best-fit (i.e., the predicted values associated with the lowest error, where error = $\Sigma$ [(observed-predicted)/(observed)]$^2$) by a model of the form:

$$C = C_1(1 - e^{-k_1 t}) + C_2(1 - e^{-k_2 t}) + \Delta \tag{3}$$

where $C$ (μmol/mg protein) is the total cation taken up by the vesicles at time $t$; $C_1$ and $C_2$ are the maximum amounts in compartments 1 and 2, respectively, at $t = \infty$; $\Delta$ is the size of an "instantaneous" compartment; and $k_1$ and $k_2$ are the first-order rate coefficients (min$^{-1}$) for compartments 1 and 2, respectively.

## Results

### Fluorescence Changes of the Voltage-Sensitive Dye, diS-$C_3$-(5)

Although a quantitative evaluation of the ability of the isolated cardiac sarcolemma membrane to develop a membrane potential has not been reported, a previous study using the voltage-sensitive probe diS-$C_3$-(5) suggested that a negative transmembrane potential difference could be established by an outwardly directed potassium gradient (Bartschat et al., 1980). Furthermore, the results of that study suggested that this technique could be useful in determination of the relative permeability characteristics of the isolated sarcolemma membrane to various monovalent cations.

A typical fluorescence recording for the protocol described in Materials and Methods is shown in Fig. 1. With the sarcolemma preparation, hyperpolarization of the membrane potential to values more negative than zero resulted in a decrease in the fluorescence intensity of the dye. The fluorescence scale was set by adjusting the instrument sensitivity to approximately 90% of full scale for the

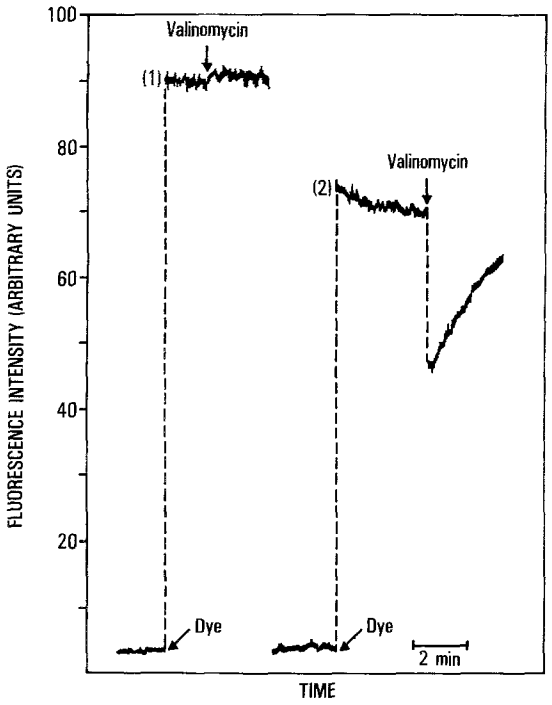


**Fig. 1.** Change in fluorescence intensity of the dye diS-$C_3$-(5) associated with sarcolemma vesicles. Aliquots of sarcolemma preparation were incubated at 4 °C with a medium containing 150 mM KCl, 10 mM NaCl and 10 mM Tris-Cl, pH 7.4, for 15 to 18 hr. Aliquots of this "loaded" suspension were then diluted into a reaction medium containing a final concentration of (1) 150 mM KCl, 10 mM NaCl and 10 mM Tris-Cl, pH 7.4 (i.e., the loading conditions) or (2) 1.5 mM KCl, 158.5 mM NaCl, and 10 mM Tris-Cl, pH 7.4, at 37 °C. After a 1 to 2 min incubation (baseline trace), the dye (1 μM final concentration) was added to the preparation, producing a rapid increase in fluorescence intensity (dashed line at dye arrow). Following approximately 2 to 4 min valinomycin (100 nM final concentration) was added in (1) and (2), delineated by the arrows. (Figure is from Hungerford, R.T., Lindenmayer, G.E., Schilling, W.R., Van Alstyne, E. 1983. The effects of membrane potential on sodium-dependent calcium transport in cardiac sarcolemma vesicles. *In:* Electrogenic Transport: Fundamental Principles and Physiological Implications. M.P. Blaustein and M. Lieberman, editors. Raven Press, New York)

condition where membrane potential would be zero (i.e., $[K^+]_i = [K^+]_o$; trace (1) in Fig. 1). It is clear from this trace that fluorescence increased after the addition of dye and remained constant for at least 2 min. Essentially no change in fluorescence intensity was observed after the addition of valinomycin. Trace (2) in Fig. 1 shows the fluorescence change of the dye for the condition where $[K^+]_i = 150$ mM and $[K^+]_o = 1.5$ mM. The fluorescence, after addition of dye but before the addition of valinomycin, was lower than that observed in trace (1). This suggests that the membrane is hyperpolarized to a value more negative than zero (inside versus outside). After about 4 min, valinomycin was added and a further decrease in fluorescence was observed. Fluorescence reached a minimum value within 10 sec and subsequently increased slowly with time. The rapid decrease in fluorescence after valinomycin was assumed to reflect further hyperpolarization of the membrane due to an increase in potassium permeability by valinomycin. The subsequent slow increase in fluorescence after the initial decrease probably reflects a change in membrane potential (i.e., depolarization) as the potassium chemical gradient was dissipated with time.

## Estimation of Membrane Potential

If the maximum decrease in fluorescence ($\Delta F$) observed after valinomycin was due to the generation of a membrane potential ($E_m$) equal to the potassium equilibrium potential ($E_K$), the relation between fluorescence intensity and external potassium should be given by:

$$\Delta F \sim E_m = E_K = \frac{RT}{F} \log \frac{[K^+]_o}{[K^+)_i} \tag{4}$$

where $[K^+]_o$ and $[K^+]_i$ are the external and internal potassium concentrations, respectively, and $R$, $T$ and $F$ have their usual meaning. A plot of $E_m$ versus log of the external potassium concentration should be linear with a slope of $-61.5$ mV at 37 °C and the intercept at the abscissa should equal $[K^+]_i$. To test if this relation holds in the sarcolemma preparation, the maximum fluorescence decrease after valinomycin (measured in arbitrary units and normalized as the percent decrease in fluorescence, $\%\Delta F$; fluorescence with zero membrane potential was set equal to 100%) was measured at different concentrations of external potassium. The $\%\Delta F$ was then equated to $E_m$ by Eq. (4) for each $[K^+]_o$ tested. For example, if the $[K^+]_o = 1.5$ mM and $[K^+]_i = 150$ mM, the $E_K$ is equal to $-123$ mV (Eq. 4). If the fluorescence change after valinomycin was equal to 64% for this condition, then the factor for converting $\%\Delta F$ to $E_m$ is 123 mV/64%$\Delta F$ or 1.923 mV/%$\Delta F$. This conversion factor was calculated for multiple values of $[K^+]_o$ and the average value obtained was used to convert all $\%\Delta F$ values to $E_m$. The results of this calibration procedure are shown in Fig. 2, open circles, where $E_m$, calculated as described above, is plotted versus log$[K^+]_o$ for the counter-cation choline. The dashed line represents the membrane potential predicted from the Nernst equation for potassium and the points are the membrane potentials calculated from measured changes in $\%\Delta F$. The slope of the regression line was equal to $-60.0$ mV and the x-intercept was

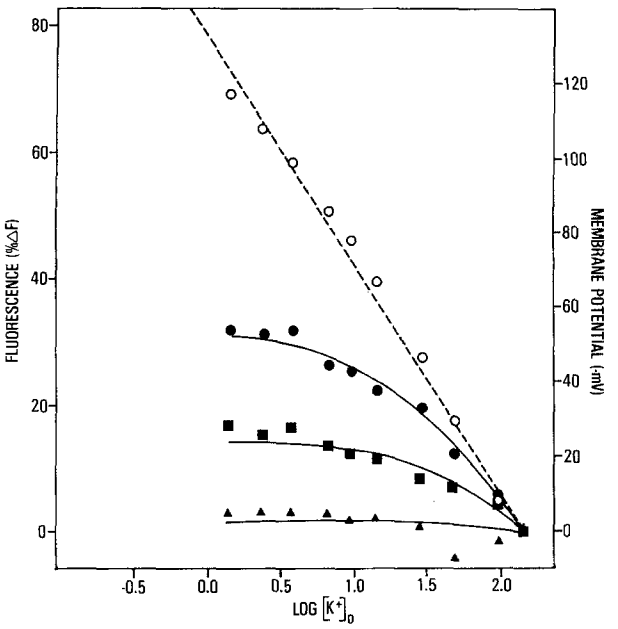


**Fig. 2.** Effect of variation in external potassium concentration on the fluorescence of the dye, diS-$C_3$-(5), (left ordinate) and $E_m$ (right ordinate) associated with sarcolemma vesicles. Aliquots of sarcolemma preparation were incubated at 4 °C with a medium containing 150 mM KCl, 10 mM counter-cation, either choline-Cl, NaCl, or LiCl and 10 mM Tris-Cl, pH 7.4 for 37 °C. Aliquots of loaded suspension were diluted into a reaction medium containing various concentrations of KCl (external potassium *plus* counter-cation concentrations was equal to 160 mM) and 10 mM Tris-Cl, pH 7.4. After a 1 to 2 min incubation; the dye (1 μM final concentration) was added to the preparation and the fluorescence recorded. After 2 to 3 min valinomycin (100 nM final concentration) was added. Solid symbols represent the $\%\Delta F$ *before* the addition of valinomycin for the counter-cations choline-Cl (●), NaCl (■), and LiCl (▲). The $\%\Delta F$ *after* the addition of valinomycin for the counter-cation choline-Cl (○) was employed to determine the $E_m$ scale as described in the text. The dashed line represents the $E_m$ predicted by the Nernst equation for potassium. Each point is the mean of experiments performed on 5 to 7 preparations each in duplicate

equal to 161.0 mM when the counter-cation was choline. Hence, an excellent correlation was obtained between the predicted and the observed membrane potentials. The data with sodium or lithium as the counter-cation deviated slightly from the Nernst prediction (*data not shown*). The x-intercept for choline was actually closer to the predicted 150 mM than the linear regression analysis suggests. There was a slight nonlinearity of dye response at very negative membrane potentials. This caused the x-intercept to be greater than 150 mM and the slope to be lower than −61.5 mV. Since the choline data most closely approximated the Nernst prediction it was used to establish the membrane potential scale in Fig. 2.

## Evaluation of Relative Permeabilities

In order to evaluate the relative permeability characteristics of the isolated sarcolemma preparation, the effect of external potassium on the fluorescence changes with choline, sodium or lithium as counter-cations in the absence of valinomycin was determined (Fig. 2, closed symbols). The data were fit to a form of the Goldman-Hodgkin-Katz (Goldman, 1943; Hodgkin & Katz, 1949) equation:

$$E_m = 61.5 \log \frac{[K^+]_o + \alpha [C^+]_o}{[K^+]_i + \alpha [C^+]_i} \quad (5a)$$

where $\alpha$ is the ratio of permeabilities, $P_C/P_K$, as determined by a single parameter optimization routine, and $P_C$ is the permeability coefficient for the counter-cation (solid lines). It is clear that the data for the three counter-cations are reasonably fit by Eq. (5a). The best fit values of $\alpha$ were 0.125, 0.301 and 0.997 for choline, sodium and lithium, respectively. These data suggested that the membrane is essentially as permeable to lithium as to potassium. In fact, at external potassium concentrations above 50 mM, it appeared that lithium has a greater permeability than potassium since positive potentials were observed. The order of permeability is K = Li > Na > choline as determined by the best fit $\alpha$ values.

The anion in these experiments was chloride and the above estimates of $\alpha$ assumed that $P_{Cl} = 0$. If chloride had a finite permeability, then Eq. (5a) must be modified to:

$$E_m = 61.5 \log \frac{[K^+]_o + \alpha [C^+]_o + \beta [Cl^-]_i}{[K^+]_i + \alpha [C^+]_i + \beta [Cl^-]_o} \quad (5b)$$

where $\beta = P_{Cl}/P_K$. If $P_{choline} = 0$, then $\alpha$ in Eq. (5b) is zero and the fitting procedure yields $P_{Cl}/P_K = 0.134$ for the case where choline-Cl is the counter-cation. If one assumes that the permeabilities of chloride and potassium are independent of the counter-cation present, then $\beta$ must also equal 0.134 for the case where sodium is the counter-cation. The fitting procedure then yields $P_{Na}/P_K = 0.202$. Thus, the data are consistent with $P_{Na}/P_K$ equal to 0.301 for $P_{Cl}/P_K = 0$ (i.e., $P_{choline}/P_K = 0.125$) and 0.202 for $P_{Cl}/P_K = 0.134$ (i.e., $P_{choline}/P_K = 0$). In fact the membrane would be expected to have finite permeabilities for both chloride and choline. Thus, $P_{choline}/P_K$ would presumably reside between 0 and 0.125, $P_{Cl}/P_K$ between 0 and 0.134 and $P_{Na}/P_K$ between 0.301 and 0.202.

Assays of a large number of sarcolemma preparations by the protocol used in Fig. 2 revealed that

while all preparations responded to valinomycin as shown, many preparations demonstrated no evidence or only minimal evidence of hyperpolarization with a potassium gradient in the absence of valinomycin regardless of the counter-cation used. This finding is of particular significance physiologically for the counter-cation sodium. The $P_{Na}/P_K$ was found to be between 0.202 and 0.301 (Fig. 2). This value is at least 10-fold higher than that observed in adult mammalian myocardium. The high $P_{Na}/P_K$ could have resulted from either a high $P_{Na}$ or a low $P_K$ value. Since most biological membranes are thought to be relatively impermeable to sodium, a high $P_{Na}$ would suggest that the sarcolemma membrane had been damaged during the isolation procedure and that this damage resulted in a membrane "leaky" to sodium. Alternatively, the potassium permeability could have been lower than observed *in vivo*. This would also result in a higher $P_{Na}/P_K$ ratio than expected. Potassium channels in nerve have been observed to be extremely labile with irreversible loss of channel function occurring when internal perfusion with zero potassium was employed (Chandler & Meves, 1970). Since the solutions used during isolation of the sarcolemma preparation contained no potassium, it was possible that the potassium channels were rendered nonfunctional with the result being a low membrane permeability to potassium. However, in two preparations which had 15 mM KCl added to the solutions used during the isolation procedure, no decrease in the $P_{Na}/P_K$ ratio was observed. In an attempt to further address these possibilities we turned to the measurement of ion fluxes for evaluation of sodium and potassium permeability coefficients.

### Optimization of Conditions for Accurate Flux Measurement

In order to define the conditions by which ionic fluxes into the isolated cardiac sarcolemma vesicles could be measured, studies were designed 1) to develop a method of stopping the tracer flux reaction under different conditions; 2) to determine the amount of time necessary for equilibration of the intravesicular space with the extravesicular space; and 3) to evaluate the effects of protein concentration on the amount of ion associated with the vesicles at equilibrium. The procedure adopted to stop the reactions was separation of the vesicles from the reaction medium by filtration followed by washes of the filter to remove extravesicular tracer. To determine how the number of washes affected vesicular tracer content after equilibrium cation uptake, vesicles were incubated with 150 mM salt with tracer ($^{42}K$ or $^{22}Na$) for 15 to 18 hr. Aliquots were removed and filtered in duplicate on a filtration apparatus. The samples were then either not washed or washed successively up to six times with 3 ml of an ice-cold *hyperosmotic* solution. In an experiment with a 100 mM KCl-loaded preparation, it was found that a *hypoosmotic* wash solution dramatically decreased the amount of cation associated with the vesicles after three washes. Therefore, the wash solution was always kept hyperosmotic with respect to the internal vesicular milieu. The amount of ion associated with the vesicles and filters dropped dramatically with the first wash (Table 1). With successive washes, the level of isotope associated with the preparation/filter dropped slowly and essentially leveled off after three washes although the trend was for a slight decline with washes greater than three in number. It should be noted that the amount of cation taken up by the vesicles after 15 to 18 hr was dependent on the cation present (potassium versus sodium).

Since the ultimate goal of this study required evaluation of time-dependent changes in cation movement, it was necessary to determine if filtration and/or washing the filter could terminate the movement of tracer at some time after the uptake reaction was initiated but before equilibrium of tracer movement was reached. After a one-minute exposure to a reaction medium containing 150 mM

**Table 1.** Cation associated with membrane vesicles/filters[a]

| Number of washes | NaCl (μmol cation/mg protein) | KCl (μmol cation/mg protein) |
|---|---|---|
| 0 | 208 ±16 | 210 ±16 |
| 1 | 3.01± 0.32 | 4.04± 0.24 |
| 2 | 2.15± 0.08 | 3.68± 0.18 |
| 3 | 1.94± 0.12 | 3.41± 0.20 |
| 4 | 1.90± 0.12 | 3.35± 0.16 |
| 5 | 1.87± 0.08 | 3.35± 0.16 |
| 6 | 1.84± 0.10 | 3.19± 0.08 |

[a] Effect of successive washes on the levels of Na and K associated with filters and vesicles: 18-hr incubation. Aliquots of sarcolemma preparation were incubated at 4 °C with a media containing 150 mM NaCl or KCl, 5 mM imidazole-Cl, pH 7.4, and tracer amounts of $^{22}Na$ or $^{42}K$. After 15 to 18 hr, essentially duplicate samples (the second sample was filtered 4 sec after the first) were filtered and the filters were either not washed or successively washed 1 to 6 times with 3-ml aliquots of an ice-cold solution containing 5 mM imidazole-Cl, pH 7.4 and either 200 mM NaCl or KCl (i.e., the salt in the loading medium). The data presented here are not corrected for the amount of radioactivity associated with the filters in the absence of protein (background). Values represent the means ± SE ($n$ = 4).

salt and tracer for the cation of the salt, the effect of filtration and successive washes on the tracer content of the vesicles/filter was determined (Table 2). Filtration plus three washes of the filter were sufficient to stop the reaction. This method of termination was used for all subsequent uptake studies.

In order to determine the amount of time necessary for equilibration of the intravesicular space with the extravesicular space, the amount of cation associated with vesicles versus time of incubation at 5 °C was measured. In these experiments the amount of salt in the incubation media was 150 mM. Sodium and potassium associated with the preparation increased rapidly over the first 90 min and plateaued by 18 hr (Table 3). Both sodium and potassium appeared to have a very slow phase of uptake between 18 and 42 hr. The 18-hr values for KCl and NaCl were 82% and 87% of the 42-hr values, respectively. However, other considerations suggested that between 15 and 18 hr of incubation is sufficient for complete equilibration of the intravesicular space with the extravesicular space for the chloride salts of sodium and potassium. As shown above, vesicles loaded overnight with 150 mM KCl in the presence of 150 mM extravesicular KCl showed minimal or no change in fluorescence intensity of the voltage-sensitive dye, diS-$C_3$-(5), upon addition of the potassium ionophore, valinomycin (Fig. 1). These preparations, however, responded in a manner consistent with the development of potassium Nernst potentials when extravesicular potassium was lower than 150 mM (Fig. 2). The slow phase of cation uptake for sodium and potassium after 18 hr may have reflected cation uptake by bacteria. Evidence for bacterial growth in the preparation after 3 to 5 days of storage at 4 °C has been obtained by inspection of the preparation with phase-contrast microscopy. Another possibility was that the slow phase actually represented true time-dependent uptake of tracer into a population of vesicles with a very low permeability to the salts. These vesicles would represent, at most, 13 to 18% of the total population and would have had a half-time for equilibration of at least 9 hr which is much longer than the time course of flux studies reported herein. For all subsequent experiments the vesicles were allowed to incubate with loading solutions for 15 to 18 hr at 4 °C before use.

The next experiment was designed to determine the effect of the amount of protein on the cation associated with the vesicles after loading. Different concentrations of the sarcolemma-enriched preparation were incubated for 15 to 18 hr in 150 mM KCl plus $^{42}K$ or NaCl plus $^{22}Na$. The chloride salts of potassium and sodium both exhibited linear increases in vesicular cation content with increasing protein filtered (Fig. 3). The $y$-intercept of the linear regression line for potassium was significantly different from zero, whereas the line for sodium passes very close to the origin. The reason for the former is unknown but probably accounts for part of the differences observed between the

**Table 2.** Cation associated with membrane vesicles/filters[a]

| Number of washes | NaCl | KCl |
|---|---|---|
| | (μmol cation/mg protein) | |
| 0 | 287 ±29 | 311 ±34 |
| 1 | 1.99 ± 0.33 | 4.04± 0.99 |
| 2 | 0.689± 0.018 | 2.38± 0.22 |
| 3 | 0.598± 0.039 | 2.20± 0.099 |
| 4 | 0.518± 0.027 | 2.16± 0.16 |
| 5 | 0.491± 0.022 | 2.20± 0.20 |
| 6 | 0.514± 0.022 | – |

[a] Effect of successive washes on the levels of Na and K associated with vesicles/filter: 1-min incubation. Aliquots of sarcolemma-enriched preparations were incubated at 4 °C for 15 to 18 hr in media containing 150 mM NaCl or KCl and 5 mM imidazole-Cl, pH 7.4. Aliquots of this suspension were added to reaction media containing 150 mM of the salt used in the loading medium, 5 mM imidazole-Cl, pH 7.4, and tracer amounts of $^{22}Na$ or $^{42}K$. After 1 min, duplicate samples were removed, filtered and either not washed or successively washed 1 to 6 times with 3-ml aliquots of ice-cold solution containing 200 mM NaCl or KCl (i.e., the salt in the loading medium) and 5 mM imidazole-Cl, pH 7.4. The total amount of radioactivity associated with the vesicles/filters was then determined. Values represent the means ± SE ($n=4$).

**Table 3.** Cation associated with vesicles[a]

| Time (hr) | KCl | NaCl |
|---|---|---|
| | (μmol cation/mg protein) | |
| 1.5 | 1.67±0.072 | 0.85±0.04 |
| 18 | 2.79±0.045 | 1.58±0.09 |
| 24 | 3.09±0.085 | 1.65±0.10 |
| 42 | 3.43±0.14 | 1.8 ±0.12 |

[a] The effect of incubation time on the levels of sodium and potassium associated with the sarcolemma-enriched preparation. Aliquots of the preparation in 5 mM imidazole-Cl, pH 7.4, were added to reaction media containing 150 mM NaCl or KCl, 5 mM imidazole-Cl, pH 7.4, and tracer amounts of $^{22}Na$ or $^{42}K$. Triplicate samples were removed at 1.5, 18, 24 and 42 hr, filtered and washed three times with 3-ml aliquots of ice-cold solution containing 200 mM NaCl or KCl (i.e., the salt in the reaction medium), and 5 mM imidazole-Cl, pH 7.4. The $^{22}Na$ or $^{42}K$ associated with the vesicles was then determined by subtracting the amount of radioactivity associated with the filters in the absence of protein (background) from the total radioactivity associated with filters and vesicles. Values represent the means ± SE ($n=4$).

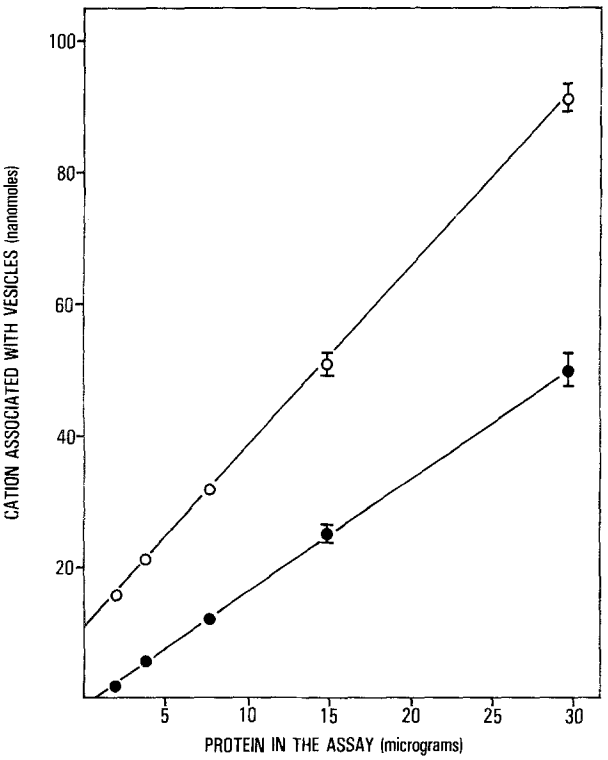


**Fig. 3.** The effect of protein concentration on the levels of sodium and potassium associated with the sarcolemma enriched preparation at equilibrium. Aliquots of the preparations containing various amounts of protein were incubated at 4 °C with media containing 150 mM NaCl (•) or KCl (o), 5 mM imidazole-Cl, pH 7.4, and tracer amounts of $^{22}Na$ or $^{42}K$. After 15 to 18 hr quadruplicate samples were removed, filtered, and washed three times with 3 ml aliquots of an ice-cold solution containing 200 mM NaCl or KCl, respectively, and 5 mM imidazole-Cl, pH 7.4. The amount of $^{22}Na$ or $^{42}K$ associated with the preparation was then determined and corrected for background radioactivity. Each point represents the mean ± SE ($n=4$); where not shown, the standard error bars are smaller than the symbols employed. Lines were from linear least-squares analysis

magnitude of sodium and potassium associated with the preparation at equilibrium (Tables 1 to 3, *see also* Fig. 5 below). It is important to emphasize that both profiles were linear between 5 and 30 μg of protein. All subsequent experiments employed protein concentrations within this range.

## Evaluation of Ionic Flux Under Nonequilibrium Conditions

Evaluation of the relative sodium to potassium permeability of the isolated cardiac sarcolemma preparation was determined indirectly by measurement of the diffusion potential [with diS-$C_3$-(5)] developed in response to an outwardly directed potassium gradient and an inwardly directed sodium gradient. The following study was designed to compare the permeability of the sarcolemma preparation to sodium and potassium by direct measurement of radiotracer fluxes under conditions similar to those employed for the dye study.

Sarcolemma vesicles, loaded with 150 mM KCl, were diluted 1:10 into a reaction media containing final concentrations of 135 mM NaCl and 15 mM KCl. Likewise, vesicles loaded with 150 mM NaCl were diluted 1:10 into a solution containing final concentrations of 135 mM KCl and 15 mM NaCl. The net efflux of potassium or sodium from the vesicles was measured by following the radiotracer $^{42}K$ or $^{22}Na$ movement out of the vesicle with time (lower curves in Fig. 4). In a parallel set of experiments (using the same preparations), the uptake of cation into the vesicles under these identical conditions was followed versus time [i.e., sodium uptake into potassium-loaded vesicles and potassium uptake into the sodium-loaded vesicles (upper curves in Fig. 4)]. The middle curve in each panel of Fig. 4 is the net cation flux; the points are the actual algebraic additions of uptake and efflux measured at each time point and the smooth curve is the algebraic addition of the best-fit exponential curves for the uptake and efflux data. For sodium-loaded vesicles, net cation movement increased in an inward direction (i.e., in the direction of net potassium flux) for approximately 3 min (panel *A*). After this time no further net cation flux was observed despite the fact that net efflux of sodium and influx of potassium continued. The same type of profile was observed for potassium-loaded vesicles (panel *B*). Net cation flux was outwardly directed (i.e., again, in the direction of net potassium flux) for approximately 8 min. Thus at earlier times, the net movement of cation was dominated by the flux of potassium, be it inwardly or outwardly directed. At later times the sodium and potassium fluxes were equal and, hence, net cation flux was in steady state. Both sets of data were consistent with the hypothesis that these sarcolemma preparations had a greater permeability to potassium than sodium. It is possible, however, that this difference resulted, at least in part, from the greater potassium content of the vesicles at time zero compared to the sodium content of the vesicles at time zero (*see* Tables 1 to 3). Since free intravesicular sodium and potassium should be the same for vesicles loaded with 150 mM sodium and potassium, respectively, the latter possibility would seem to require that the amount of cation adjacent to the internal membrane surface (rather than free cation in the intravesicular space) is a major determinant of the magnitude of cation flux.

The apparent steady state of net cation movement seen in Fig. 4 could be the result of concen-

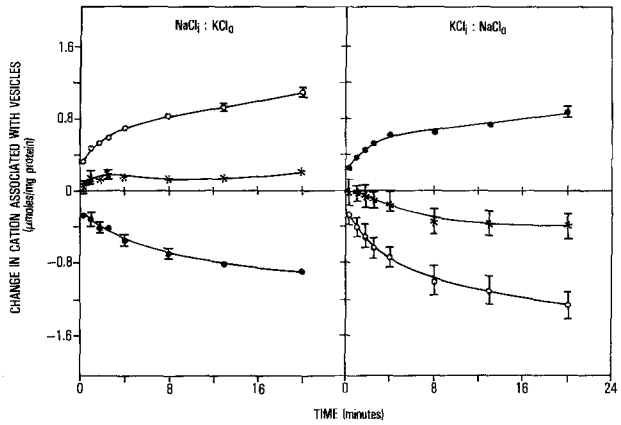


**Fig. 4.** Time courses for $Na^+$, $K^+$, and net cation movement associated with sarcolemma vesicles: nonequilibrium conditions. Aliquots of sarcolemma preparation incubated at 4 °C for 15 to 18 hr in media containing 150 mM NaCl or KCl, and 5 mM imidazole-Cl, pH 7.4, were diluted 1:10 into reaction media containing a final concentration of 135 mM NaCl, 15 mM KCl or 135 mM KCl, 15 mM NaCl and 5 mM imidazole-Cl, pH 7.4. For each panel the upper curve represents net cation uptake in a medium containing tracer amounts of $^{22}Na$ or $^{42}K$, and the lower curve represents net movement of cation out of vesicles "loaded" with tracer amounts of $^{22}Na$ or $^{42}K$. Duplicate samples (times 4 sec apart) were filtered at succeeding times and washed three times with 3 ml-aliquots of ice-cold solution containing 200 mM NaCl, or KCl (i.e., the salt in the loading medium), and 5 mM imidazole-Cl, pH 7.4, and the $^{22}Na$ or $^{42}K$ associated with the vesicles was determined. For net outward movement of $^{22}Na$ or $^{42}K$, the amount of radioactivity associated with the vesicles at zero times was determined by two methods: 1) aliquots of loaded suspensions were diluted 1:10 into reaction media containing the loading salts in concentrations and specific activities identical to the loading media, and quadruplicate samples were filtered and washed; or 2) quadruplicate samples of the loaded suspension were filtered directly; no significant difference was found between these two methods. These "zero points" were then subtracted from the data points at successive times to obtain the values for net outward movement. The movements of $^{22}Na$ (●) and $^{42}K$ (○) were measured concurrently in the same four preparations under conditions described above. The net cation or difference data (⋆) were determined for each preparation. Each point represents the mean of four preparations, ±SEM; where not shown, the standard error bars were smaller than the symbol employed. Upper and lower smooth curves represent the best fit predictions for the mean data for a one- or a two-compartment exponential model; middle curves are the difference between the predicted curves; "*i*", the intravesicular salt, "*o*" the extravesicular

tration-dependent changes in membrane permeability to sodium and/or potassium as the ionic gradients dissipated with time. Alternatively, chloride flux could have been influencing cation flux, or the steady state could represent a balance between membrane potential, changes in ionic concentration gradients and membrane cation permeabilities. Net chloride fluxes were measured under conditions identical to those employed for the cation fluxes in Fig. 4. Essentially, no movement of chloride occurred between 17 sec and 20 min. Membrane potential was also measured under conditions identical to those described in Fig. 4 by use of the potential sensitive dye, diS-$C_3$-(5). The fluorescence change observed suggested that the membrane potential was close to zero and remained constant for 20 min. The latter experiments were performed on three other sarcolemma preparations loaded separately with KCl or NaCl. Results from all of these experiments suggested that the membrane potential in the net cation flux studies in Fig. 4 was close to zero. This explained why little chloride movement was observed. Since the chloride concentration was equal inside and outside the vesicle and the potential was equal to zero, chloride was in electrochemical equilibrium and no net flux would be expected. It seems likely, therefore, that neither membrane potential nor chloride movements were affecting the profiles for cation flux. However, because of these potential complications in the interpretation of the ion flux data, the next set of experiments was designed to determine the permeability coefficients for sodium and potassium under equilibrium conditions.

## Cation Uptake in the Absence of a Net Electrochemical Driving Force

In principle, the membrane permeability coefficients for potassium and sodium can be determined by measuring the rate coefficients of tracer cation uptake versus time under equilibrium conditions (i.e., concentrations of salt in intravesicular and extravesicular spaces are equal). Under these conditions sodium or potassium influx is given by (Sperelakis, 1979):

$$J_{Na} = P_{Na}[Na^+]_o \text{ or } J_K = P_K[K^+]_o \qquad (6)$$

where $P_{Na}$ and $P_K$ are the permeability coefficients (cm $sec^{-1}$) for sodium and potassium, and $[Na^+]_o$ and $[K^+]_o$ are the extravesicular sodium and potassium activities (moles $cm^{-3}$). To obtain flux (mole $cm^{-2}$ $sec^{-1}$), the rate coefficients ($k$, in $sec^{-1}$) from uptake experiments (*see below*) were used according to:

$$J_{Na} = k_{Na}V/A[Na^+]_o \text{ or } J_K = k_K V/A[K^+]_o \qquad (7)$$

where $V$ = volume ($cm^3$) and $A$ = surface area ($cm^2$) of the vesicles (estimated for idealized spherical vesicles from average diameter values obtained from electron microscopy; $V/A = 1.67 \times 10^{-6}$ cm; Van Alstyne et al., 1980). A plot of $J_{Na}$ or $J_K$ versus sodium or potassium activities under these condi-

tions should be linear with a slope equal to the permeability coefficient. Thus, $P_{Na}$ and $P_K$ are functions of the rate coefficient and, according to these equations, should be independent of ion concentrations.

## Effect of Salt Activity on Cation Uptake

The effects of cation activity in the incubation medium on the amount of cation associated with the vesicles at equilibrium was determined. Aliquots of the preparation were incubated for 15 to 18 hr with different concentrations of KCl or NaCl with $^{42}K$ or $^{22}Na$. Cation concentrations were corrected to activities as described under Materials and Methods. As cation activity in the incubation medium increased, the amount of cation associated with the vesicles increased in a linear fashion up to a salt activity of 143 mM (Fig. 5). At 178 mM salt, a deviation from linearity was observed. This deviation may be due to alterations in membrane structure induced by the high ionic strength.

If one assumes that all of the cation associated with the vesicles was distributed in the intravesicular water space, an apparent volume of distribution can be calculated at each cation activity. At low cation activities the volumes of distribution for both potassium and sodium were large relative to values obtained at higher activities (Fig. 6). Over the range of about 60 to 143 mM salt, little change in the volumes of distribution was observed. At greater activities, however, a decrease was found. Qualitatively, the profiles for sodium and potassium were the same, but the vesicles seemed to possess a greater capacity for potassium accumulation than for sodium. This suggested that the intravesicular water space was greater in the presence of KCl than NaCl. The chloride water space for the two salts was found to be approximately 7.5 µl/mg protein. Thus, not only was the chloride volume of distribution the same for sodium and potassium but it was lower than that observed for these cations. This strongly suggests that the volume of intravesicular bulk solution does not differ for NaCl and KCl and that some other mechanism must account for the different apparent volumes of cation distribution. It is important to emphasize that the amount of sodium and potassium associated with the sarcolemma preparation at equilibrium was linear over a wide range of concentrations (Fig. 5). This range of concentrations was employed in subsequent experiments for the evaluation of sodium and potassium permeability coefficients.

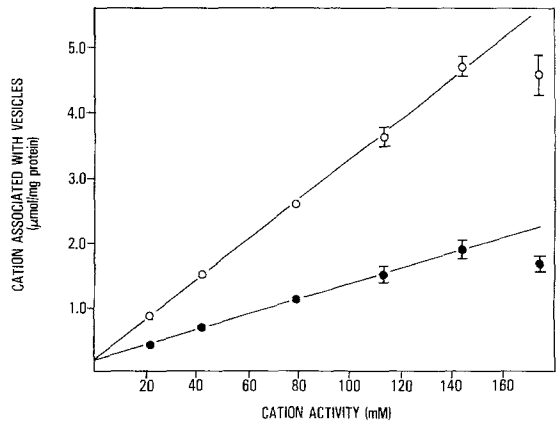


**Fig. 5.** The effect of sodium and potassium activities on the levels of sodium and potassium associated with the membrane vesicles at equilibrium. Aliquots of the sarcolemma preparation were incubated at 4 °C with media containing NaCl (●) or KCl (○) in concentrations ranging from 25 to 250 mM (activities calculated as described in Materials and Methods), 5 mM imidazole-Cl, pH 7.4, and tracer amounts of $^{22}Na$ or $^{42}K$. After 15 to 18 hr quadruplicate samples were removed, filtered, and washed three times with 3-ml aliquots of ice-cold solution containing 250 mM NaCl or KCl, respectively, and 5 mM imidazole-Cl, pH 7.4. The $^{22}Na$ or $^{42}K$ associated with the vesicles was then determined. Each point represents the mean ± SE ($n=4$); where not shown, the standard error bars are smaller than the symbol employed. Lines were drawn from linear least-squares analysis excluding the observations for cation activity of 178 mM

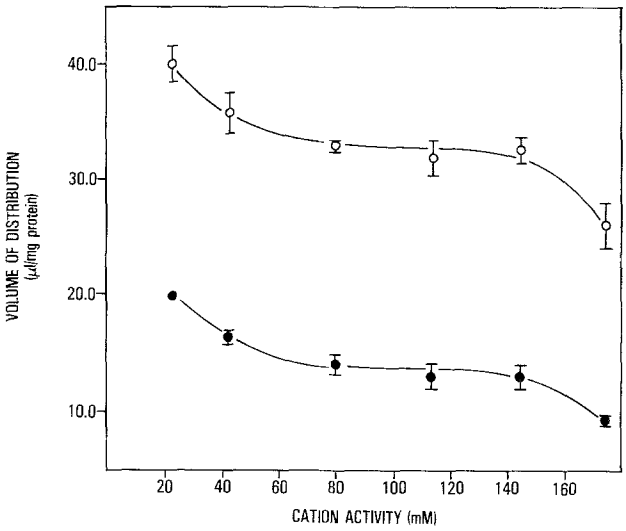


**Fig. 6.** The effect of sodium and potassium activities on the apparent volume of distribution of sodium and potassium associated with the sarcolemma-enriched preparation at equilibrium. The apparent volume of distribution at each activity was calculated (µmol/mg protein ÷ µmol/µl = µl/mg protein) from the data presented in Fig. 5 assuming that 1) the intravesicular activity is equal to the extravesicular activity (at equilibrium), and 2) the total amount of $^{22}Na$ or $^{42}K$ associated with the vesicles is in the bulk solution within the vesicles. Each point represents the mean ± SE ($n=4$)

## Evaluation of Cation Permeability Coefficients

The sarcolemma-enriched preparation was equilibrated for 15 to 18 hr with various concentrations of KCl and NaCl. Aliquots of the loaded preparation were then rapidly added to solutions containing the salt concentration identical to that in the loading medium and tracer $^{42}K$ or $^{22}Na$. The intravesicular salt concentration was assumed to equal the extravesicular concentrations and the membrane potential was assumed to be zero (i.e., the ions present were in electrochemical equilibrium). The uptakes of potassium and sodium over 17 sec to 90 min followed exponential time courses (Fig. 7). These experiments were repeated over a range of salt concentrations from 10 to 200 mM. At all concentrations tested, the best-fit model was found to be the bi-exponential plus instantaneous compartment model (Eq. 3). Note, however, that the data in Table 3 indicate that the amount of cation associated with the preparation at 1.5 hr is only 50 to 60% of the values observed at 18 hr (which presumably represents complete equilibration). Despite the fact that these experiments were performed on preparations different from those for Table 3 (*see below*), this suggests that one or more components of cation flux may be expressed at longer times. Cation flux using both light scatter techniques (Schuil, 1981) and radiotracer flux techniques (Schilling, 1981; Schuil, 1981) at 37 °C was evaluated in other experiments. In many cases the light scatter was followed for 4 to 6 hr and the radiotracer fluxes at 37 °C were followed out to 80 to 90% of the 15 to 18 hr equilibration value. The data at 37 °C were also consistent with the conclusion that the sarcolemma preparation manifests two major components of cation flux. It re-

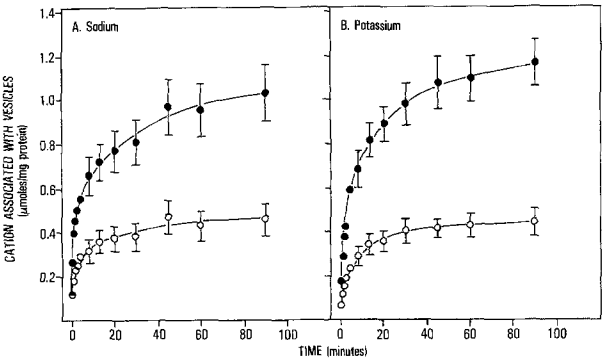


**Fig. 7.** Time courses for sodium and potassium uptake by the sarcolemma-enriched preparation under equilibrium conditions. Aliquots of the preparation were incubated at 4 °C in media containing various concentrations of NaCl or KCl, 10 mM Tris-Cl, pH 7.4, for 15 to 18 hr. The loaded suspensions were then added to reaction media containing NaCl or KCl in concentrations equal to those in the loading media, 10 mM Tris-Cl, pH 7.4, and tracer amounts of $^{22}Na$ or $^{42}K$. The reactions were terminated by filtration and the filters were washed three times with 3-ml aliquots of ice-cold solution containing 200 mM NaCl or KCl (i.e., the cation in the loading medium), 10 mM Tris-Cl, pH 7.4. *Panel A*: 50 mM (○) and 150 mM (●) NaCl. *Panel B*: 50 mM (○) and 150 mM (●) KCl. Each point represents the mean ± SE ($n = 4$ to 6) except that error bars were omitted from the initial points for clarity. Curves derive from best-fit of the mean data by text Eq. (3)

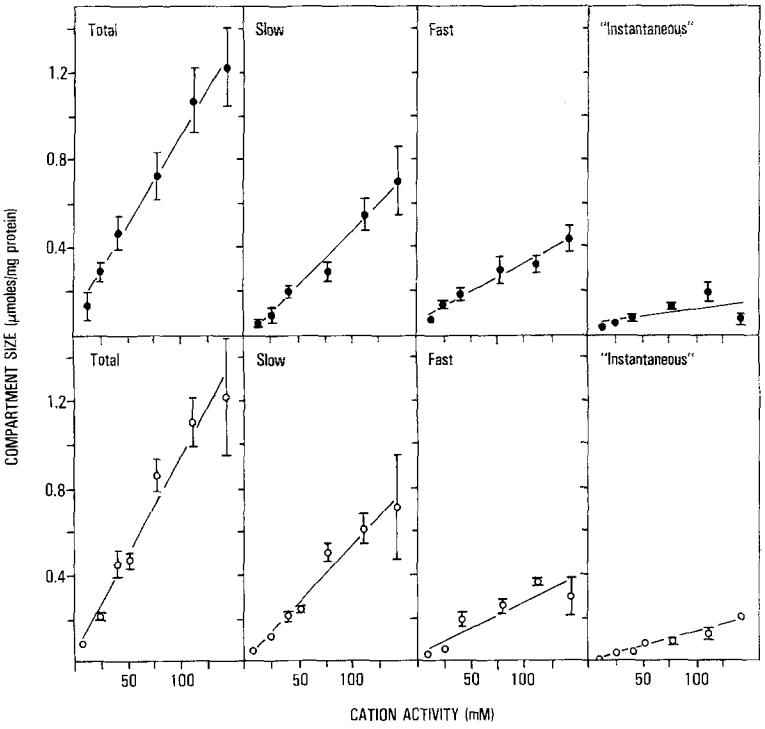


**Fig. 8.** Compartment sizes for sodium and potassium uptake under equilibrium conditions. The $^{22}Na$ and $^{42}K$ uptake data for a number of concentrations (examples shown in Fig. 7) were analyzed according to text Eq. (3) ("Instantaneous" = $A$; Slow = $C_1$; Fast = $C_2$; Total = sum of the three individual compartments, i.e., $TC = C_1 + C_2 + A$). Upper panels NaCl (●); lower panels KCl (○). The data points for 10 mM KCl represent the averages of two preparations. All other values represent the means ± SE ($n = 3$ to 6); where not shown, the standard error bars were smaller than the symbol employed. Lines were drawn from linear least-squares analysis

mains possible, however, that one or more slower components of cation flux may be expressed at longer times at 5 °C.

Compartmental analysis revealed similar profiles for sodium and potassium (Fig. 8). In general, the compartment sizes were linear functions of cation activity over the concentration range examined, perhaps indicating that these compartments represent the low affinity, high capacity environment of the vesicle interior. It should be noted that the "instantaneous" compartment was also a linear function of cation activity, saturating or deviating from this relationship only at 200 mM NaCl. This result suggested that the latter compartment could reflect a very rapid, exponential process for which no data points could be obtained by manual mixing techniques.

In contrast to the marked differences observed between vesicular sodium and potassium at equilibrium (Fig. 5, Tables 1 to 3), no differences were observed for this set of experiments. However, it should be noted that these experiments were performed on different sets of preparations and at different times. The equilibrium sodium content of the vesicles after a 150 mM NaCl load varied between 0.939 and 2.21 μmol per mg protein for 35 preparations over an eleven-month period. In contrast, the equilibrium potassium content of the vesicles was found to vary between 1.20 and 3.32 μmol per mg protein for 24 preparations over an eight-month period. The mean ± SE equilibrium amounts of cation associated with the vesicles for all preparations studied was $1.48 \pm 0.052$ and $2.19 \pm 0.11$ μmol/mg protein for sodium and potassium, respectively.

As described above, the rate coefficient for uptake at a particular ion concentration or activity can be used to calculate the magnitude of ion flux. A plot of ion flux versus cation activity should be linear with a slope equal to the permeability coefficient of the membrane for the cation. The results of this analysis are shown in Fig. 9 for the two major compartments of cation uptake (fast and slow), where flux was calculated as described using the rate coefficients derived from the compartmental analysis. The slow component of sodium flux (Fig. 9; panel *A*) was linked in a linear manner to increases in cation activity. Likewise, the fast component of potassium flux (panel *D*) was characterized by a linear profile over the entire range of activities tested. Thus, in both of these cases, the flux could be described by a concentration-independent permeability coefficient; $P_{Na}$ (for the slow compartment) is equal to $1.77 \times 10^{-9}$ cm/sec and $P_K$ (for the fast compartment) is equal to $1.1 \times 10^{-8}$ cm/sec at 5 °C. These values are in

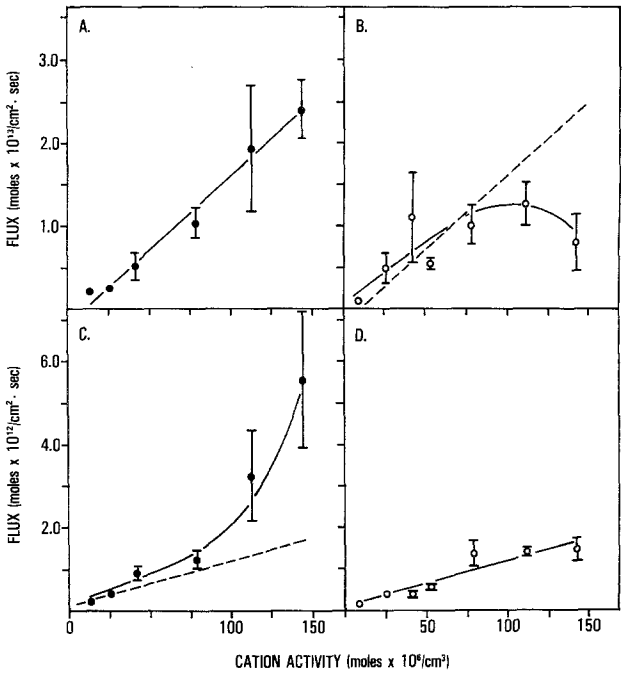


**Fig. 9.** Effect of extravesicular cation activity on the calculated cation flux across vesicles in the sarcolemma-enriched preparation. The $^{22}Na$ and $^{42}K$ uptake data for a number of activities (examples shown in Fig. 7) were fit to text Eq. (3). The rate coefficients ($k_1$ for the slow compartment and $k_2$ for the fast compartment) generated from these analyses were used to calculate the net cation fluxes for each cation activity from the text Eq. (7). The upper panels represent the slow compartment and the lower panels represent the fast compartment from the analyses for sodium (•) and potassium (○); note the differences in scale on the ordinate. Solid lines in panel *A* and panel *D* were drawn from linear least-squares analysis. These lines are presented as broken lines in panels *B* and *C*, respectively, for comparison. The slopes of these regression lines should represent the cation permeability coefficients (cm/sec), since $P = kV/A$ (text Eqs. 6 & 7); in panel *A*, $P_{Na} = 1.77 \times 10^{-9}$ cm/sec; in panel *D*, $P_K = 1.1 \times 10^{-8}$ cm/sec. Solid lines in panel *B* and panel *C* were fit by eye. The data points for 10 mM KCl represent the means of two preparations. All other points represent the means ± SE ($n = 3$ to 6); where not shown the standard error bars were smaller than the symbol employed

the range of those reported for the sodium permeability coefficient of the myocardial cell but are at least 10-fold lower than estimates of the potassium permeability coefficient. Panels *B* and *C* show the effects of cation activity for the slow component of potassium flux and the fast component of sodium flux, respectively. At low activities, these fluxes approached those observed for the other cation into the same compartment (dashed line in Panels *B* and *C* are the same as solid lines in *A* and *D*, respectively), but at higher activities deviations were observed. The fast component of sodium flux increased at higher activities while the slow component of potassium flux decreased. The deviations from linearity may have reflected either ionic strength effects or concentration-dependent

changes in the cation permeability coefficients. Since the slow component of potassium flux decreased at higher activity, this profile could also be explained, in part, by saturation of potassium flux at higher potassium activities.

## Discussion

Evaluation of the relative membrane permeability characteristics of the isolated cardiac sarcolemma vesicle was accomplished by two different techniques. The results of both the potential-sensitive dye and the radiotracer flux studies were consistent with a relatively high $P_{Na}/P_K$ ratio, and the measurement of equilibrium sodium and potassium fluxes generated the conclusion that $P_K$ is lower in the isolated sarcolemma preparation than that for the intact cardiac cell.

The high $P_{Na}/P_K$ ratio could have been the result of a generalized damage of the sarcolemma membrane during the isolation procedure. Evaluation of the change in light scatter of the sarcolemma preparation following a hyperosmotic challenge to a variety of inorganic salts (including NaCl and KCl) and nonionic compounds has suggested that the permeability of the sarcolemma preparation to sodium and potassium is intermediate on a continuum between urea on the more permeable extreme and sucrose or glucose on the less permeable extreme (Schuil, 1981). In agreement with the light scatter results, measurement of [$^{14}$C]sucrose uptake has suggested that this preparation is essentially impermeable to sucrose (Schilling, 1981). These findings are inconsistent with generalized damage of the isolated cardiac sarcolemma membrane. Furthermore, the radiotracer flux studies reported herein suggested that while sodium permeability approximated the value obtained for the cardiac cell, the potassium permeability is lower, again suggesting that nonspecific damage during the isolation procedure did not occur.

The first set of radiotracer experiments presented in this study were designed to optimize the conditions necessary for the accurate measurement of cation movement into the sarcolemma vesicles. It was found that for all of the experimental conditions employed, filtration and three, 3 ml washes with ice-cold hyperosmotic salt solution could decrease the amount of cation associated with the vesicles to a value that essentially did not change with additional washes. After washing and correction for tracer associated with the filter, the remaining radioisotope should have represented: 1) cation bound to extremely high affinity sites on the outer surface of the membrane (which seems unlikely); 2) cation within the membrane (i.e., possibly trapped in aqueous channels which presumably is a very small amount); 3) cation in the internal diffuse double layer; and 4) cation present in the bulk intravesicular water space. The volume of distribution of the cations was nearly constant over the activity range of approximately 50 to 143 mM. At activities lower than about 50 mM, the volume of distribution increased suggesting that a significant amount of cation binding occurred, possibly reflecting attraction towards fixed negative charges on the inner surface of the membrane. This binding apparently saturated at cation activities higher than about 50 mM salt since the volume of distribution for the cations remained constant between 50 and 143 mM.

The differences observed between the sodium and potassium volumes of distribution after 15 to 18 hr incubation could result from either an actual change in the volume of the vesicle or from a difference in the binding of the two cations to the negatively charged surface of the vesicle membrane. The chloride volumes of distribution were measured under identical conditions for sodium and potassium salts. It was found that the chloride volume of distribution did not depend on the cation present, but rather, was the same for both sodium and potassium. Thus, the differences observed between sodium and potassium could not be due to changes in the volume of the vesicle, which suggests that potassium was concentrated to a greater extent than sodium in the internal diffuse double layer. Chloride would be expected to be excluded from the diffuse double layer (McLaughlin, 1977). If chloride is excluded to the same extent that sodium and potassium are accumulated, a difference in the chloride volume of distribution between the sodium and potassium salts would be expected. Since no difference was seen, the chloride volume of distribution may have predominantly reflected chloride in the bulk intravesicular water space.

As might be expected for a fragmented membrane preparation, the movements of sodium and potassium into the vesicles were characterized by multi-exponential processes. Similar results were found with light scatter experiments which followed the decay of an initial response to osmotic challenges (Schuil, 1981). Two major compartments for cation movement were observed in the present study. The compartment sizes were found to increase as linear, nonsaturable functions of the cation activity. An obvious interpretation of these profiles is that two populations of vesicles exist in the sarcolemma preparation, one of which has greater permeability to solutes than the other. It should also be noted that vesicles of different size

with similar inherent permeabilities can yield different apparent permeabilities, since the permeability coefficient ($P$) for ion movement across the membrane is a function of the vesicular volume-to-surface area ratio (Eqs. 6 and 7); for a sphere, which the vesicles appear to approximate (Van Alstyne et al., 1980):

$$P = k_{\text{cation}} \frac{r}{3} \tag{8}$$

where $k_{\text{cation}}$ is equal to the apparent rate coefficient for cation movement and $r$ is equal to the radius of the vesicle. If two vesicles had the same permeability coefficient and differed only in size, then a 10-fold difference in radii between the two vesicles would be required in order to realize a 10-fold difference in rate coefficients as observed in the present study. Although estimates of vesicle sizes in this preparation have suggested a narrow range of radii from 25 to 75 nanometers (Van Alstyne et al., 1980), calculations suggest that a normal distribution or a skewed distribution of vesicle size (where all vesicles have the same density of flux pathways) would also produce flux results consistent with a two-compartment fit.

It is possible that the two compartments of cation flux reflect the presence of right-side-out and inside-out vesicles that were formed in the fragmentation (homogenization) procedure. The ratio of the "slow" to the "fast" component is about 2:1 to 3:1 (Fig. 8). Activation of ($Na^+$, $K^+$)-ATPase activity by freeze-thaw cycles, which renders the vesicles permeable, was about three- to four-fold. The number of specific ouabain sites was increased 30 to 40% by a similar intervention (Van Alstyne et al., 1980). Thus, it is reasonable to conclude that the "slow" component reflects sealed right-side-out vesicles while the "fast" component, which equilibrates with cations at a 10- to 20-fold greater rate, reflects inside-out vesicles that are inherently more permeable to the cations or that are skewed towards a smaller size (Eq. 8). Alternatively, the two populations could reflect subfactionation of the sarcolemma membrane from different areas of the cell surface (e.g., vesicles from transverse tubules; vesicles from surface membrane). Different areas of the sarcolemma might be expected to have unique permeability characteristics.

From Eq. (6) the unidirectional cation flux under conditions of equal intra- and extravesicular cation concentrations should be related in a linear manner to the extravesicular cation activity by the permeability coefficient for the cation. The sodium and potassium flux versus cation activity agreed only in part with these predictions. Sodium flux into the slow compartment did vary linearly with the sodium activity which yielded $P_{\text{Na}}$ equal to $1.77 \times 10^{-9}$ cm/sec. Potassium flux into the fast compartment also varied linearly with the potassium activity which gave a value for $P_{\text{K}}$ of $1.1 \times 10^{-8}$ cm/sec. In marked contrast, sodium flux into the fast compartment and potassium flux into the slow compartment showed nonlinear profiles with increasing cation activity. Thus, $P_{\text{Na}}$ and $P_{\text{K}}$ were not constant over the activity range employed. The sodium permeability coefficient for the slow compartment appeared to be an upper limit for the potassium permeability coefficient for this compartment. Conversely, the potassium permeability coefficient for the fast compartment appeared to be a lower limit for the sodium permeability coefficient for that compartment (Fig. 9).

Because of the differences observed between sodium and potassium flux it seems reasonable to postulate the presence of at least two pathways for cation movement across the membrane of the isolated cardiac sarcolemma vesicle. The first pathway is operative at lower cation activities, saturates at higher activities, and does not discriminate between sodium and potassium. The second pathway appears to be relatively selective for sodium, activating at the higher sodium activities. This movement may reflect sodium/sodium exchange. Activity of this nature has been reported in an isolated cardiac sarcolemma preparation and has been postulated to occur through the sodium/calcium exchanger (Reeves & Sutko, 1979). Where the experimental design precluded detection of one-to-one sodium/sodium exchange (i.e., dye study, Fig. 2; equal specific activities of $^{22}Na$ in the intra- and extravesicular spaces, Fig. 4), the data suggest that the membrane permeability for potassium is equal to or slightly greater than that for sodium.

Colquhoun et al. (1981) measured single-channel currents from heart muscle cells with the patch clamp technique. The channels, which were stated to be abundant, demonstrated little selectivity between sodium and potassium and exhibited low permeability to chloride. These characteristics are similar to those observed for the sarcolemma vesicle preparation used in the present study. Coronado and Latorre (1982) measured the conductances of three different types of potassium channels from purified heart sarcolemma vesicles, after incorporation into planar lipid bilayers. One type of channel did not discriminate between sodium and potassium; a second had a $P_{\text{Na}}/P_{\text{K}}$ ratio equal to 0.33; the third had a $P_{\text{Na}}/P_{\text{K}}$ ratio of 0.2. Hence, a relatively high $P_{\text{Na}}/P_{\text{K}}$ ratio may be a general characteristic of sarcolemma isolated from myocardial tissue.

Assuming a $Q_{10}$ of 1.5 for the rate coefficient

for flux, the permeability coefficients of the slow compartment for sodium agreed well with the *in situ* permeability coefficient of myocardial cells for sodium (Carmeliet et al., 1976). This generates the conclusion that the potassium permeability coefficient of this compartment is much lower than the $P_K$ *in situ*. The permeability coefficient of the fast compartment for potassium was about 10-fold greater than the *in situ* sodium permeability coefficient, but was still four to seven fold lower than the estimates of $P_K$ in heart (Carmeliet et al., 1976; Carmeliet & Verdonck, 1977; Horres, Aiton & Lieberman, 1979). It should be pointed out that the steady-state potassium conductance for intact cardiac tissue is thought to vary with ionic composition of both the external (Noble, 1965; Vassalle, 1965; Lee & Fozzard, 1979) and internal milieu (Isenberg, 1977). Caroni and Carofoli (1982) have recently reported an effect of calcium on potassium permeability in the isolated cardiac sarcolemma preparation. Also, Galper et al. (1982) reported that muscarinic agonists can increase potassium permeability in cultured heart cells. In light of this, it remains to be determined if the potassium permeability in the sarcolemma preparation is inherently low or is in a low conductance state as a result of the absence of one or more physiological regulators.

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