Activities of Potassium and Sodium Ions in Rabbit Heart Muscle

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ABSTRACT Activities (a) of intracellular K and Na in rabbit ventricular papillary muscles were determined with cation-selective glass microelectrodes and concentrations (C) were estimated with flame photometry. The C_K and a_K of the muscles were 134.9 ± 3.1 mM (mean value ± SE) and 82.6 mM, respectively, at 25°C. The corresponding C_{Na} and a_{Na} were 32.7 ± 2.7 and 5.7, respectively. The apparent intracellular activity coefficients for K (γ_K) and Na (γ_{Na}) were 0.612 and 0.175, respectively. Similar results were obtained at 35 ± 1°C. γ_K was substantially lower than the activity coefficient (0.745) of extracellular fluid (Tyrode's solution), which might be expected on the basis of a different intracellular ionic strength. γ_{Na} was much lower than that of extracellular fluid, and suggests that much of the Na was compartmentalized or sequestered. For external K concentrations greater than 5 mM, the resting membrane potentials agreed well with the potential differences calculated from the K activity gradients across the cell membrane as a potassium electrode. These results emphasize that potassium equilibrium potentials in heart muscle should be calculated by activities rather than concentrations.

INTRODUCTION

To understand the electrophysiological properties of excitable cells, it is highly desirable to know the intracellular ion activities rather than their bulk concentrations. Realizing the structural complexity and macromolecular content of the cells, it is difficult to assume that the cellular water and electrolytes are homogeneously distributed and are in the free state, as in a simple electrolyte solution.

Cation-selective glass microelectrodes have been used for measurement of intracellular Na and K activities in cells such as *Carcinus* muscle fibers (Hinke, 1959), *Loligo* giant axons (Hinke, 1961), frog skeletal muscle (Lev, 1964), single proximal tubules of *Necturus* kidney (Khuri et al., 1963), and toad oocytes (Dick and McLaughlin, 1969). The apparent Na activity coefficients a_{Na}/C_{Na}) in these tissues are about 0.2–0.3, which indicates that a major portion of the Na is not free in the cytoplasm. This low intracellular Na ac-

tivity is further supported by the physiological experiments of Thomas (1972) in snail neurones using "recessed-tip" Na-sensitive microelectrodes. On the other hand, the ratio of a_K/C_K for intracellular K is reported to be close to the activity coefficient of solutions bathing the cells, a rather surprising result considering the high cell content of multivalent anions.

Measurements of the intracellular ion activities with cation-selective glass microelectrodes have been usually limited to large cells because of the size and the high resistance of the microelectrodes. With modifications of construction techniques, the physical dimensions of working tip of the microelectrodes were reduced so that they could be applied to relatively small cells (Lee and Armstrong, 1972), and tip resistances of the microelectrodes were lowered markedly by aging in 3 M KCl solution (Lee and Fozzard, 1974 *a*). Such microelectrodes gave the stable potentials that are required to obtain accurate ion activities in the small cells.

In the present studies the activities of Na and K in the papillary muscle cells of rabbit heart bathed in normal physiological solution were measured with cation-selective glass microelectrodes. For comparison, the Na and K contents were determined with flame photometry using sulfate space as a measure of the extracellular compartment. This allows calculation of "apparent activity coefficients" for these ions, but it should be recognized that this method of measuring cellular Na content is subject to large errors because of Na binding to extracellular structures and difficulties in measurement of extracellular space. Also, the ionic activities in the muscle cells under different K concentrations of the bathing solution were measured with the cation-selective glass microelectrodes. Some preliminary observations have been reported (Lee and Fozzard, 1974 *b*).

METHODS

Experimental Procedure

Papillary muscles from rabbit left ventricles were used in the experiments. The animals were stunned by a blow on the neck. The heart was quickly removed and rinsed free of surface blood with oxygenated Tyrode's solution at room temperature (25°C), and two left ventricular papillary muscles were removed (wet weight about 40–60 mg). For determination of total contents of Na and K one muscle was equilibrated for about 3 h in 20 ml of oxygenated Tyrode's solution containing a trace amount of ^{35}S. Total water was then measured by drying to constant weight for 24 h at 105°C. ^{35}S, Na, and K of the muscle were extracted into 4 ml of 0.1 N HNO_3 by agitating for about 72 h at room temperature. Two aliquots of 1 ml of this extract were used to estimate extracellular volume by counting ^{35}S with a Nuclear-Chicago Mark I liquid scintillation counter (Nuclear-Chicago, Des Plaines, Ill.). The rest of 0.1 N HNO_3 extract was used to measure total contents of Na and K. The extracts were diluted with a solution containing lithium as an internal standard and analyzed for Na and K by

emission spectrophotometry (Instrument Laboratory, Inc., West Seattle, Wash., model 143).

The other papillary muscle was used for measurement of transmembrane potentials. The muscle was immersed in a perfusion chamber containing oxygenated Tyrode's solution (pH 7.2) at 25°C. Membrane potential was measured with conventional open-tip microelectrodes filled with 3 M KCl and with tip resistances of 20–50 MΩ and tip potentials less than 5 mV. At the same time penetrations on different cells of the muscle were also made with a pair of sealed cation-selective glass microelectrodes with different selectivity coefficients. These microelectrodes were calibrated before and after the membrane potential measurements with standard test solutions containing NaCl and KCl of 1, 10, and 100 mM as described elsewhere (Lee and Fozzard, 1974 *a*). The whole sequence of electrical measurements required about 3 h to complete. Thereafter, total water, Na, and K contents of this muscle were analyzed for comparison with those of the muscle immersed in ^{35}S-contained Tyrode's solution. If the total contents of Na and K were not close to each other, the experiments were discarded. Similar experiments were made with fibers at 35°C.

In another set of experiments, the intracellular K and Na activities were measured at the different membrane potentials that were achieved by a decrease or increase of the external K concentration in the Tyrode solution. The K concentrations were 2, 5, 10, 30, and 50 mM, and were varied by interchange of KCl for NaCl so that the external solutions contained constant Cl concentration of 150 mM. Right ventricular papillary muscles (diameter of about 1 mm) were equilibrated in the Tyrode solution or test solution about 30 min before measuring membrane potentials and intracellular K and Na activities.

Determination of Intracellular Na and K Activities

The construction of the microelectrodes used in this study was similar to that described previously (Lee and Armstrong, 1974). The microelectrodes must meet stringent requirements for use in heart cells, because cell diameters are on the order of 10 μm. The inner sealed micropipettes made from $NAS_{27\text{-}04}$ glass (Corning Glass Works, Corning, N.Y.) had tip diameters of less than 1 μm. The exposed tip lengths were less than 3 μm. The tip diameters of outer insulating micropipettes made from Kimax glass tubes (Kimble Products, Div., Owens-Illinois, Inc., Toledo, Ohio) were about 1 μm. When the exposed tip length was greater than 3 μm or the tip diameter of outer insulating micropipette was greater than 1 μm, unstable or low membrane potentials were obtained.

Electrochemical properties of the microelectrodes have already been reported (Lee and Fozzard, 1974 *a*). The resistance of the microelectrodes was greater than 10^9 Ω immediately after they were made. The high resistance could be reduced to the order of 10^7 or 10^8 Ω by aging the microelectrodes in 3 M KCl for a few days or more before insulation, which is also accompanied by a change in the selectivity coefficient (k_{KNa}).

The potentials (E_K) measured with a given K-selective glass electrode in Na-K mixture solutions can be represented according to the following equation.

$$E_K = E_o + S \ln (a_K + k_{KNa}\, a_{Na}), \quad (1)$$

where E_o is a constant of the electrode; S represents an empirical slope of the calibration curve of a microelectrode ($\Delta E_K/\Delta \log a_K$), varying from 55 to 61 mV; a_K and a_{Na} are activities of K and Na in the test solution; k_{KNa} is the selectivity coefficient for the electrode. On the basis of Eq. 1, intracellular Na and K activities were determined by two methods that employed different assumptions.

(*a*) When a microelectrode potential is measured in the external bathing solution, Eq. 1 can be represented as follows:

$$E_K^o = E_o + S \log (a_K^o + k_{KNa}\, a_{Na}^o), \tag{2}$$

where E_K^o is potential measured with a given microelectrode in the bathing solution; a_K^o and a_{Na}^o are K and Na activities in the bathing solution, respectively.

When the microelectrode penetrates into a cell from the bathing solution, Eq. 1 can be written in the following form,

$$E_K^i - V_M = E_o + S \log (a_K^i + k_{KNa}\, a_{Na}^i), \tag{3}$$

where E_K^i is potential measured with the microelectrode with respect to the reference electrode in the bathing solution; V_M is transmembrane potential measured with conventional open-tip microelectrodes; a_K^i and a_{Na}^i are intracellular K and Na activities, respectively.

Subtraction of Eq. 2 from Eq. 3 gives the following equation:

$$a_K^i + k_{KNa}\, a_{Na}^i = (a_K^o + k_{KNa}\, a_{Na}^o) e^{(\Delta E - V_M)/S}, \tag{4}$$

where ΔE, $(E_K^i - E_K^o)$ is the change in potential when the microelectrode is penetrated into a cell from the bathing solution. By this method one avoids the need for E_o as in the second method, but K and Na activities in the external solution are needed.

In individual experiments, advantage was taken of the fact that different microelectrodes made from the same glass can have different selectivity coefficient (k_{KNa}). For each muscle used, when a pair of microelectrodes with different selectivity coefficients is chosen, the following two equations are obtained from Eq. 4.

$$a_K^i + k'_{KNa}\, a_{Na}^i = (a_K^o + k'_{KNa}\, a_{Na}^o) e^{(\Delta E' - V_M)/S'}. \tag{5}$$

$$a_K^i + k''_{KNa} a_{Na}^i = (a_K^o + k''_{Na}\, a_{Na}^o) e^{(\Delta E'' - V_M)/S''}. \tag{6}$$

Two electrodes with quite different selectivity coefficients were chosen for each experiment and experiments were divided into two groups; one with electrodes of lower selectivity coefficient and the other with electrodes of higher selectivity coefficient. $\Delta E'$ and $\Delta E''$ are the average values of potential changes when the microelectrodes with lower and high selectivity coefficients, respectively, are penetrated into cells.

(*b*) The second method is similar to that described elsewhere (Lev, 1964; and Lee and Armstrong, 1974).

RESULTS

Potential Difference Measurements

After 30-min incubation, resting membrane potentials were measured with an open-tip microelectrode. The membrane potentials were accepted only if they remained constant within 1 mV for at least 1 min (upper trace, Fig. 1). The potential measurements were not accepted if the tip potentials changed more

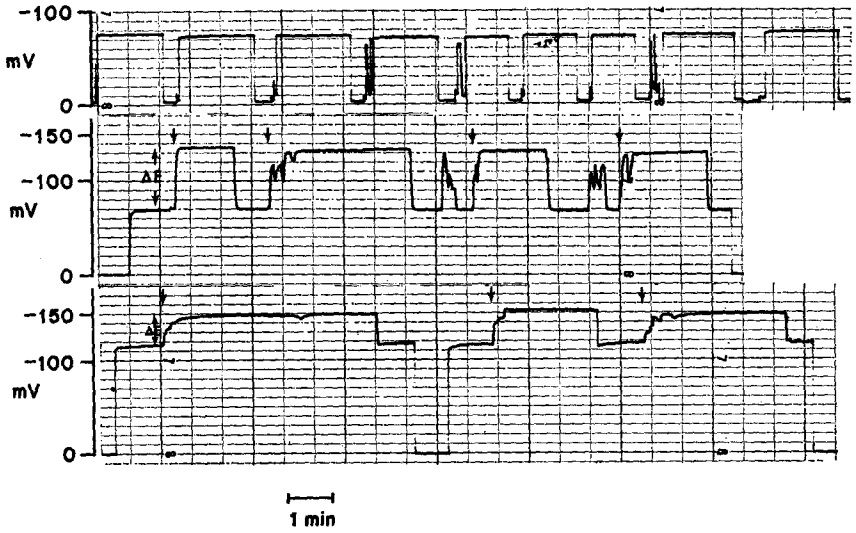

FIGURE 1. Recordings of transmembrane potentials. Upper tracing: resting membrane potentials measured with an open-tip microelectrode. Middle tracing: transmembrane potentials measured with a cation-selective glass microelectrode with higher selectivity coefficient. The unsuccessful impalements between the second and third successful impalements or the third and fourth successful impalements indicate that the microelectrode tip glanced off cells or that seal of cell membrane around the microelectrode was not satisfactory. Lower tracing: transmembrane potentials measured with a cation-selective glass microelectrode with lower selectivity coefficient.

than 1 mV from penetration to withdrawal. As seen in the tracing, the tip potentials were less than -5 mV for each membrane potential measurement. Thereafter, at least four impalements of individual cells were made with a calibrated cation-selective glass microelectrode to obtain ΔE of Eq. 4. Middle and lower tracings of Fig. 1 show examples of typical recordings of these potential measurements with microelectrodes with a different selectivity coefficient. The middle tracing shows four successive potential measurements with the cation-selective microelectrode. It was observed that, as long as the potential registered by the withdrawn microelectrode in the bathing solution remained within 1 mV of that before impalement, the characteristics of the microelec-

trode remained the same and recalibration was not necessary after each series of potential measurements. In this way ΔE of Eq. 4 was obtained as the average value of potential changes when a microelectrode entered from bathing solution into a cell. The experiment was accepted if the resting membrane potentials remained virtually constant throughout the experiments. Table I shows the results of resting membrane potentials (V_M) and potential changes (ΔE) at 25°C. A mean value of -75.2 ± 1.1 mV was obtained from 98 resting membrane potential measurements from eight muscles immersed in Tyrode's solution. The mean values of $\Delta E'$ and $\Delta E''$ were -45.0 ± 0.6 mV and -61.3 ± 0.7 mV, measured with the cation-selective microelectrodes with lower (group I) and higher (group II) selectivity coefficients, respec-

TABLE I

DATA FOR CALCULATION OF INTRACELLULAR K AND NA ION ACTIVITIES AT 25°C

	V_M	ΔE	k_{KNa}	S
	mV	*mV*		*mV*
Group I		−45.0±0.6 (n=42)	0.195±0.003	59.8±0.2
	−75.2±1.1 (n=98)			
Group II		−61.3±0.7 (n=38)	0.386±0.009	56.4±0.3

Resting membrane potential (V_M; mean value ± SE; n, number of measurements) of papillary muscle cells. Potential difference (ΔE; mean value ± SE; n, number of measurements) measured with cation-selective glass microelectrodes across the cell membrane. Selectivity coefficient (k_{KNa}) and slope (S) of eight microelectrodes for each group (mean value ± SE).

tively. The slopes of calibration curves ($S = \Delta mV/\Delta \log a_i$) were 59.8 ± 0.2 mV and 56.4 ± 0.3 mV for the microelectrodes with lower and higher selectivity coefficients, respectively. Lower and higher selectivity coefficients of the microelectrodes were 0.195 ± 0.003 and 0.386 ± 0.009, respectively, for eight electrodes of each group. Determination of S and k_{KNa} was described previously (Lee and Fozzard, 1974 *a*).

Na and K Activities in Tyrode's Solution

As required in Eqs. 5 and 6, the Na and K activities of Tyrode's solution were needed for the determination of intracellular Na and K activities. These activities can be calculated from the activity coefficient and their concentrations in the solution, or they can be measured with cation-selective glass microelectrodes. The activity coefficient of the solution calculated according to Debye-Hückel equation (Robinson and Stokes, 1965) is 0.745. E_K of Eq. 2 for determination of a_{Na} and a_K was measured with a pair of cation-

selective glass microelectrodes used in this study. Tyrode's solution contained 153.0 mM Na and 5.4 mM K. The calculated and measured activities for Na were 114.0 mM and 112.7 ± 1.8 mM (mean value ±SE n = 8), and the corresponding values for K were 4.0 mM and 4.1 ± 0.2 mM. The calculated activities are in good agreement with those measured with the microelectrodes.

Activities of K and Na Ions in Papillary Muscle Cells

Table II summarizes the results obtained with left ventricular papillary muscle immersed in Tyrode's solution at 25°C. The results, labeled A in the table were obtained by the first method for calculation of intracellular Na and K activities. The intracellular Na activity, a_{Na}, and Na content referenced to

TABLE II

SUMMARY OF RESULTS OBTAINED WITH LEFT VENTRICULAR PAPILLARY MUSCLE IMMERSED IN TYRODE'S SOLUTION

	C_{Na}	a_{Na}	γ_{Na}	C_K	a_K	γ_K	Temperature
	mM	*mM*		*mM*	*mM*		°C
A	32.7±2.7	5.7	0.175	134.9±3.1	82.6	0.612	25
	32.7±2.3	6.4	0.205	136.1±2.9	83.4	0.613	35
B	32.7±2.7	6.3	0.193	134.9±3.1	82.2	0.609	25

Sodium and potassium concentrations (C), activities (a), and activity coefficients (γ), in rabbit papillary muscle cells (C_{Na} and C_N ; mean value standard error). The results, A and B, were obtained by the first and second method, respectively.

apparent cell water, C_{Na}, were 5.7 mM and 32.7 ± 2.7 mM, respectively. This concentration and activity yield an apparent sodium activity coefficient of 0.175. This result is similar to those reported (Lev, 1964; Kostyuk et al., 1969; Armstrong and Lee, 1971) for frog skeletal muscles, and is much lower than would be predicted on the assumption that all the apparent intracellular sodium is in homogeneous solution.

The intracellular potassium activity, a_K, and apparent concentration, C_K, were 82.6 mM and 134.9 ± 3.1 mM, respectively. The calculated apparent molar activity coefficient for K is 0.612, and it is significantly different from those obtained from frog skeletal muscles. Lev (1964) measured the K ion activity and concentration in frog skeletal muscle fibers and obtained the value of about 0.75 for the activity coefficient, very close to that of Ringer's solution. An essentially similar value, about 0.74 for the K activity coefficient, was reported in the same tissue (Armstrong and Lee, 1971). Therefore it has been concluded that most of the intrafiber K ions in the skeletal muscle cells are in the free state, as in a KCl solution with similar ionic strength. On the

other hand, the apparent activity coefficient for K in the papillary muscle cells is different from that predicted for this ion in free solution under similar conditions.

In order to seek temperature effects on the intracellular ion activities, Na and K activities of the papillary muscles were measured by identical procedures, with correction of the electrode constants at 35 ± 1°C. At this temperature the intracellular ion activities were calculated by the first method only. As shown in Table II the activities and calculated activity coefficients of intracellular Na and K ions are similar to those obtained at 25°C.

B of Table II shows the results obtained by the second method of calculation. The activities and activity coefficients were obtained from the same muscles used in the first method. The intracellular Na and K activities of 6.3 and 82.2 mM are very close to those obtained by the first method. Walker and Ladle (1973) obtained intracellular K activity of 86.2 mM in frog ventricular muscle by liquid ion-exchanger microelectrodes. However, they did not attempt to estimate the ionic activity coefficient.

Calculation of cytoplasmic activity coefficients requires the measurement of concentrations of sodium and potassium in the cells. One source of error in this measurement is determination of the extracellular volume with sulfur-35-labeled SO_4 ions. It was observed that with this substance the volume of distribution reached a steady level after 3-h immersion of 32.9 ± 2.4 (SE) ml/100 g wet wt and did not increase significantly (at the 95% confidence level) during a further 2-h immersion period. While it seems unlikely that SO_4 space is a true measure of the extracellular Na and K space (see Discussion), it represents a standard for comparison with other studies. The error from this source should be small for K, but could be large for Na. For this and other reasons, the estimation of cytoplasmic Na content is erroneously high, resulting in an excessively low Na activity coefficient.

Effects of External K Concentrations on Transmembrane Potentials in the Steady State

In the preceding experiments the intracellular K activity coefficient was substantially lower than that of the external solution. The usual method of calculation of the potassium equilibrium potential uses concentrations and would be in error. Actually calculated K equilibrium potentials across the cell membrane of cardiac muscles exceed the membrane potentials measured with conventional microelectrodes even in high external K concentrations (Weidmann, 1956; Page, 1962). The differences between the two potentials increase with lowering the external K concentration. In this experiment, the intracellular K and Na ion activities of right ventricular papillary muscles were measured and the membrane potentials calculated from the intracellular and extracellular K ion activities were compared with the membrane potentials measured with conventional microelectrodes (Table III).

TABLE III

MEASUREMENT OF K AND NA ION ACTIVITIES AND COMPARISON OF MEMBRANE POTENTIALS

$[K]_o$	a_K	a_{Na}	$V_M \pm S.E.$	V_{a_K}	V_K	No. of muscles
mM	*mM*	*mM*	*mV*	*mV*	*mV*	
2	81.7	7.3	−89.1±0.9	−102.6	−108.1	8 (n=103)
5	83.1	6.4	−77.5±0.5	−79.5	−84.6	7 (n=71)
10	82.9	5.8	−61.9±0.4	−61.7	−66.8	8 (n=68)
30	83.6	6.3	−33.3±0.6	−33.7	−36.6	7 (n=72)
50	83.4	5.9	−21.4±0.8	−20.5	−25.5	5 (n=58)
Mean ± SE	82.9±0.1	6.3±0.3				

Intracellular K (a_K) and Na (a_{Na}) activities, resting membrane potentials (V_M), and potentials calculated with intracellular and extracellular K activities (V_{a_K}) and concentrations (V_K) at different $[K]_o$. n is number of measurements of membrane potential.

The intracellular K and Na ion activities of the muscles equilibrated in the external solutions containing 2, 5, 10, 30, and 50 mM K were relatively constant. The mean values of 82.9 and 6.5 mM for the intracellular K and Na ion activities are in good agreement with those (Table II) of the left ventricular muscle cells immersed in normal Tyrode's solution. The resting membrane potentials are lower than the equilibrium potentials calculated from the internal and external K concentration in which a value of 135 mM for the internal K concentration was used. The difference increases with lowering the external K concentration and is about 17 mV when the external solution contained 2 mM K. However, the membrane potential is close to that calculated from the intracellular and extracellular K activities for each $[K]_o$ if $[K]_o > 5$ mM. These results are illustrated in Fig. 2. It is obvious that the difference between the two lines is due to the difference in the intracellular and extracellular ion activity coefficients. In these experiments, the product of K and Cl concentration was not kept constant. This was felt to be unimportant because of the low Cl permeability of heart cell membranes, and some control experiments support this conclusion.

Sources of Error

A number of problems could have introduced errors into the measurement of ionic activities. The ion-sensitive glass must be insulated from the extracellular solution while exposed to the cytoplasm, a problem emphasized by the pH experiments of Carter et al. (1967). Our use of a glass insulating pipette required the introduction into the small cardiac cells of a tip as large as 1 μm in diameter. The insulating micropipette could have damaged the cell membrane so that incomplete sealing occurred, resulting in a shunt that would lower the membrane potential in the region of the ion-sensitive electrode. The require-

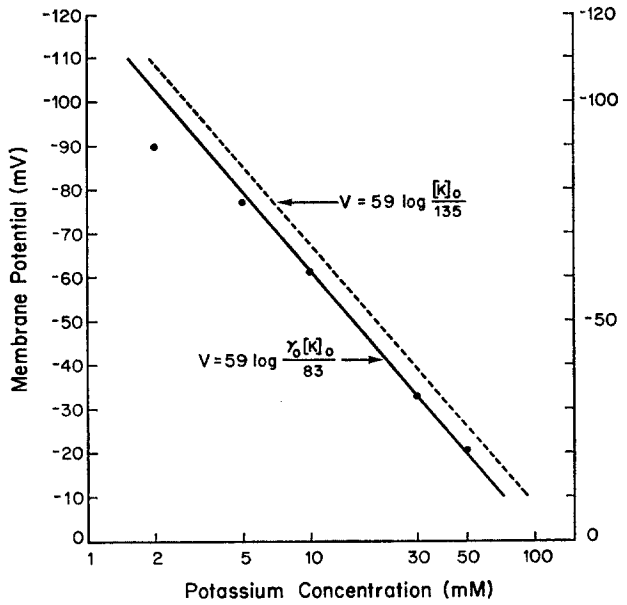

FIGURE 2. Relation between resting membrane potential and log $[K]_o$. The filled circles represent the measurement of membrane potentials after 30-min equilibration. The solid line represents the equation for a potassium electrode using measured activities and the dotted line represents the calculation using concentrations. The values of 135 and 83 in the equations are the intracellular K concentration (mM) and activity (mM), respectively. γ_o is the activity coefficient of external bathing fluid (0.75).

ment that the potential be stable for 1 min and that repeated impalements give the same value reduces, but does not completely rule out, the possibility that significant shunting occurred. If the recorded potential was less negative because of the shunt than it should have been, the apparent ion activity and activity coefficient would be erroneously high. In order to test the possible shunt, resting membrane potentials were measured with insulating micropipettes similar to those used in this study. The resting membrane potential was -68.0 ± 1.2 (SE, $n = 20$), about 6 mV less than that measured with conventional microelectrodes. If this 6-mV loss of potential is also present when the central ion-specific electrode is in place, the error introduced would be less than 15% of a_K and a_{Na}. The fact that measured activity coefficient for potassium was lower than in Tyrode's solution suggests that a shunt error was not large.

DISCUSSION

State of K in Cardiac Muscle Cells

The interesting point in this investigation is the fact that the apparent activity coefficient for K ions in the rabbit papillary muscle is significantly lower

(0.612) than that predicted for this ion in the free solution bathing the muscle cells (0.745), a result different from skeletal muscle. The ionic activity (a_{is}) of i ion that is sequestered in the cells can be estimated by the following equation:

$$a_{is} = C_i \cdot \gamma_i - a_i, \tag{7}$$

where C_i and a_i are the intracellular concentration and activity of i ion, as determined by flame photometry and cation-selective microelectrodes, respectively. γ_i is the activity coefficient of i ion in the cells which is assumed to be equal to that (0.745) in the bathing Tyrode's solution. When the values C_K = 134.9 mM and a_K = 82.6 mM (Table II) are inserted in Eq. 7, the sequestered potassium activity in the muscle cells (a_{Ks}) is found to be 18 mM, referenced to cell water. When the activity of 18 mM K is converted into concentration, 24 mM K, about 18% of the total intracellular potassium concentration is obtained. Therefore, it is suggested that a significant fraction of the intracellular potassium in the cardiac cells is sequestered.

A part of the sequestered K could result from coulombic forces, with a lower K activity coefficient in the cytoplasm. Indeed, considering that much of the cellular anion is organic and multivalent, ionic strength should be higher than the outside solution and ionic activity would be lower. In addition, deviation from values predicted by the Debye-Hückel equation would be expected for a complex solution containing Na, K, Mg, phosphocreatine, ATP, and other charged molecules. In view of this reasoning, it is surprising that measurements on skeletal muscle (Lev, 1964) and nerve fibers (Hinke, 1961) suggest that most of the cellular K is in free state in the fiber water. The correspondence between the intracellular and extracellular activity coefficients for skeletal muscle could be to some extent fortuitous, and cannot be by itself taken as direct proof that all of the fiber K is free. Recent studies (Lee and Armstrong, 1974) on frog skeletal muscles have provided some direct evidence for a significant fraction of complexed K and have indicated that this fraction of the fiber K may play an important role in net Na-K exchange.

There are other possible explanations for the low K activity compared to content measured by flame photometry. K could be bound to membranes or to macromolecules in the cytoplasm. Carvalho and Leo (1967) reported that the lipoprotein membranes of the sarcoplasmic reticulum isolated from skeletal muscle of rabbit have a K-binding capacity. K could be compartmentalized at high concentration in cellular organelles such as mitochondria. In heart muscle of the rat, Page et al. (1971) reported that the mitochondrial volume fraction was 0.34. This relatively high content of mitochondria compared to skeletal muscle might explain the difference in the two tissues. Finally, it should be pointed out that an error in evaluating the amount of intracellular K might be in the relationship between a solute and a solvent. If there is a finite fraction of nonsolvent water in the cells, the activity coefficient of the

intracellular ions calculated here would be higher than the actual activity coefficient.

If a significant fraction of intracellular K is sequestered, K activity should be used rather than its concentration in the calculation of membrane potential difference. In cardiac cells the membrane potentials calculated from intracellular and extracellular K concentrations exceed the membrane potentials measured with microelectrodes, and the difference is accentuated by low external K. In this study -82.6 mV and -75.2 ± 1.1 mV were obtained for calculated and measured membrane potentials, respectively, at $[K]_o$ of 5.4 mM. The transmembrane potential calculated from the intracellular and extracellular K^+ activities was -77.0 mV, which is close to the measured potential. These results suggest that the difference between -82.6 and -77.0 mV may be due to the sequestered or bound K in the myocardial cells. This suggestion is supported further by the results obtained from the right ventricular muscles equilibrated at different concentrations of external K. For $[K]_o > 5$ mM the measured resting membrane potentials are quite close to the theoretical equilibrium potentials obtained by the intracellular and extracellular K activities (Fig. 2 and Table III). At $[K]_o$ of 2 mM the resting potential deviated from the theoretical line for a potassium electrode. This deviation could be explained by the decrease in relative K permeability of the cell membrane or the contribution of relative Na permeability to the membrane potential at this low $[K]_o$. In addition, the relationship might be achieved by the proper combination of depolarization due to passive Na entry and offsetting hyperpolarization due to electrogenic Na pumping, with postulation of the necessary voltage dependency of these variables.

State of Na in Cardiac Muscle Cells

When the intracellular Na concentration and activity of 32.7 and 5.7 mM are inserted into Eq. 7, the sequestered Na activity in the muscle cells is found to be 18.7 mM. When the Na activity of about 19 mM is converted into concentration, 26 mM, about 79% of the total intracellular Na concentration is obtained. Thus, one is led to conclude that a major portion of the intracellular Na is sequestered and is also not included in the extracellular Na measured by conventional means, the equilibrium distribution of $^{35}SO_4$ in all the extracellular space. These results are similar to those of earlier studies on skeletal muscle (Lev, 1964; Kostyuk et al., 1969; Armstrong and Lee, 1971).

Several possible interpretations can be considered to explain this low Na activity in the muscle cells. First, as discussed elsewhere (Lee and Armstrong, 1974), some Na is probably bound to connective tissue, as is seen in skeletal muscle (Harris and Steinbock, 1956), or to sarcoplasmic macromolecules, such as myosin. Second, Na ions could be compartmentalized at a high concentration within intracellular organelles such as sarcoplasmic reticulum and

mitochondria. Evidence for such compartmentalization in organelles in rat skeletal muscle (Rogus and Zierler, 1973) and other tissues (Dick et al., 1970) has been reported. Third, Na ions could be located in extrasarcoplasmic structures, specifically the central elements of T systems, which are in direct communication with the exterior of the cells (Caldwell, 1968). The extracellular region may not be included in estimates of the extracellular space obtained by conventional methods. If we assume that the central elements are not included in the extracellular space and have the same concentration with the extracellular fluid, 0.012-vol fraction of the central elements (Page et al., 1971) has only a very small effect on the total myoplasmic Na concentration. It is clear that a substantial amount of the sequestered Na is extracellular, and is incorrectly attributed to the cytoplasm because of extracellular Na binding and difficulty in measurement of the extracellular space. If this error were as large as 10 mM, then the Na activity coefficient would be about 0.30. Probably the remainder of the apparent cellular Na is compartmentalized and not free in the cytoplasm.

In support of the low Na activity in heart muscle are the experiments of Keenan and Niedergerke (1967), who found 8–8.5 mM Na/Kg cell water in frog ventricular muscle, a tissue that has scanty sarcoplasmic reticulum. There appears to be general agreement that cytoplasmic Na activity is low compared to Na content of the tissue, whether the result of binding or compartmentalization. Thus, it is probably necessary to use Na activities rather than concentrations for calculation of Na equilibrium potential and for studies on the Na pump in muscle.

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