Potassium in Dog Ventricular Muscle: Kinetic Studies of Distribution and Effects of Varying Frequency of Contraction and Potassium Concentration of Perfusate

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The goal of a current series of investigations1-3 is to establish the basic characteristics of ionic exchange in the mammalian myocardium and to relate these characteristics to the function of the muscle. It has been evident since studies such as those of Daly and Clark4 that ionic movements cannot be considered independently, one from the other. The characteristics of calcium exchange, the influence of sodium concentration on this exchange and its attendant effect on mammalian myocardial function have been demonstrated.2 These studies led to further investigation of the effect of increases in frequency of contraction upon calcium exchange and to the hypothesis that shifts of sodium within the tissue might represent the basic control of calcium exchange and thereby one of the mechanisms for the regulation of myocardial contractility.3 This hypothesis cannot be tested further until more is known of the characteristics of sodium and potassium exchange in the experimental preparation used in these studies, namely, in the isolated, perfused papillary muscle of the dog. Potassium exchange is the subject of the present investigation.

Studies on atrial,5-7 ventricular8 or whole heart preparations9-13 in a variety of species have yielded conflicting results with respect to certain basic aspects of potassium exchange. Much of the diversity stems undoubtedly from species and tissue differences as well as differences in perfusion technique. The present study defines potassium exchange in the same tissue under the same perfusion conditions employed previously for the definition of calcium exchange.1-3 This should allow for future comparison and correlation of ionic movements with a minimum of extrapolation and interpolation.

Methods

For these experiments we used the isolated, arterially perfused dog papillary muscle preparation as described previously in detail.1,2 Adult mongrel dogs of either sex weighing 10 to 15 kg were anesthetized with pentobarbital (30 mg/kg) iv. Following administration of 75 to 100 mg of sodium heparin intravenously the dogs were sacrificed by overdosage of pentobarbital and the heart was rapidly excised. The artery of a papillary muscle from the right side of the interventricular septum was cannulated and the muscle mounted as described.1,2 From this mounted muscle it was possible to record a number of parameters continuously and simultaneously: (1) frequency of contraction with the rate controlled, when desired, by means of stimulation through platinum electrode loops attached to either end of the muscle; (2) isometric systolic and diastolic tensions with a Schilling isometric transducer;14 (3) isotopic activity of the muscle as monitored by a small G-M probe (model 222A Geiger tube, Atomic Access., Inc., Valley Stream, New York), the window of which was placed...
within 2 cm of the side of the muscle. The radiation to the window of the probe from the muscle was collimated by a lead cylinder of 1.8 cm length and 1 cm wall thickness; (4) isotopic activity of the venous effluent during washout of isotope from the previously K\(^{42}\)-labeled muscle. The standard perfusion fluid had the following composition: NaCl, 130 mM; KCl, 4 mM; CaCl\(_2\), 5 mM; NaHCO\(_3\), 14 mM; Na\(_2\)HPO\(_4\), 0.435 mM; MgCl\(_2\)·6H\(_2\)O, 1.0 mM; and glucose, 5.56 mM, and was used for all experiments except those in which the concentration of KC1 was intentionally altered. The perfusion fluid was equilibrated to 98% O\(_2\)-2% CO\(_2\) at 24°C and contained approximately 0.04 ml O\(_2\)/ml at a pH of 7.3 to 7.4 at the 150 mm Hg perfusion pressure used. Perfusion rate was approximately 1 ml/g tissue/min.

For isotopic labeling of the tissue, the perfusion fluid contained K\(^{42}\) (supplied by Union Carbide, Oak Ridge, Tennessee or Iso/Serve Inc., Cambridge, Massachusetts) to provide specific activities between 12 and 25 μc/ml at start of perfusion. All readings were corrected every 10 minutes throughout the course of the experiment for radioactive decay of K\(^{42}\). One muscle was also labeled simultaneously with K\(^{42}\) and with sucrose-C\(^{14}\). The perfusion fluid contained labeled sucrose (supplied by International Chemical and Nuclear Corporation, City of Industry, California) at a specific activity of 12.5 μc/ml.

If venous effluent was analyzed for activity during isotopic washout, the effluent droplets were collected in individual planchets. The isotopic activity of each droplet was counted and plotted semilogarithmically. The curves were analyzed graphically according to the method described by Solomon.33 Following perfusion the wet weight of the muscles was determined. The muscles were then dried in porcelain crucibles at 90 to 100°C until weight stable (four to six hours) and the percentage tissue water calculated. The tissue was then digested in concentrated HNO\(_3\) and the digestant distributed uniformly in planchets for counting of total isotopic activity.

Total tissue potassium at various perfusion times was derived from analysis of multiple samples from right ventricular wall (prior to perfusion) and from septal tissue after one, two, three, and four hours perfusion. Analyses were done on 442 samples from 22 hearts. After percent tissue water was found, the samples were ashed at 600°C for 12 hours. The ash was then dissolved in ion-free H\(_2\)O and analyzed for potassium content by flame spectrophotometry (model B flame spectrophotometer, Beckman Instruments, Inc., Palo Alto, California).

**Results**

**K⁺ CONTENT OF PERFUSED MYOCARDIUM**

Figure 1 indicates the pattern of net K⁺ loss and fluid accumulation which occurred in myocardial tissue perfused at 23 to 24°C with a cell-free and protein-free medium over the course of four hours. Net K⁺ loss was rapid for the first two hours of perfusion, but thereafter decreased markedly. Net loss of K⁺ from tissue in four hours amounted to approximately 50% of control K⁺ when corrected for the increase in total tissue water. Since dissection, mounting, and isotopic labeling of a papillary muscle required approximately three hours, the further net loss of myocardial potassium and the increase in tissue fluid were both very slight during the period when the kinetics of K\(^{42}\) washout were being defined. Depletion of tissue K⁺ prior to washout was present, however.

**KINETICALLY DEFINED PHASES OF K⁺**

A portion of the semilogarithmic plot of tissue and effluent activity during washout of...
K\textsuperscript{18} from a papillary muscle, isotopically labeled for 60 minutes and washed out for 70 minutes, is illustrated in figure 2. Graphical analysis of the effluent curve indicates that it may be resolved into three exponentially defined phases (0 to 2). This basic pattern was defined in 18 muscles labeled for periods varying between 31 and 85 minutes and washed out for periods of 40 to 100 minutes. It is noteworthy that the phase 2 tissue and effluent plots were parallel in the washout illustrated. This indicates that all of the tissue K\textsuperscript{+} which had been labeled in 60 min was accounted for in the phases defined during washout.

The following phase rate constants (\(\lambda\)) were defined (respective time constants, \(\tau\), in parentheses) in muscles contracting at an average 8 beats/min.

\[
egin{align*}
0 &= 3.2 \pm 0.2^* \text{ min}^{-1} \quad (\tau = 0.31 \text{ min}) \\
1 &= 0.65 \pm 0.02 \text{ min}^{-1} \quad (\tau = 1.54 \text{ min}) \\
2 &= 0.0139 \pm 0.0006 \text{ min}^{-1} \quad (\tau = 72 \text{ min})
\end{align*}
\]

These three phases do not, however, account for all of the myocardial potassium. In a papillary muscle which was labeled with isotope for a prolonged period (194 min) a slight, but definite, divergence between tissue and effluent plots was evident in the subsequent washout until the 180th min. This is illustrated in figure 3. At 180 min both curves deviated from the previously established exponential and tended to become parallel with a markedly decreased slope. This indicates the presence of phase 3 with a relatively slow exchange rate (< 0.004 min\(^{-1}\)).

Anatomically, intracellular potassium would be expected to exchange with the vascular system in a series arrangement through the interstitial space. Under the kinetic condi-

\*1 SE of the mean.
POTASSIUM KINETICS IN VENTRICULAR MUSCLE

Exchanges imposed by this "open series two compartment system," the phase exchange constants, \( \lambda_1 \) and \( \lambda_2 \), are accurate representations, respectively, of interstitial and cellular exchange, \( K_1 \) and \( K_2 \), if flux at the capillary is sufficient to prevent the development of a concentration gradient in the interstitium. If such a gradient develops then \( \lambda_1 \) and \( \lambda_2 \) do not accurately represent the true exchange constants, \( K_1 \) and \( K_2 \), but each is a composite of \( K_1 \) and \( K_2 \) in varying proportion depending on the magnitude of the gradient. The following ratios will determine the existence of a concentration gradient:

\[
\frac{K_2}{K_1} = \text{cellular exchange constant/interstitial exchange constant}
\]
\[
\frac{C_iV_i}{C_oV_o} = \text{cellular ionic content/interstitial ionic content}
\]
\[
\frac{A_i}{A_o} = \text{cellular surface area/capillary surface area}
\]

These ratios are made up of components of the basic efflux equation, \( m_e = KC_iV/A \). The smaller each ratio, the more accurately will \( \lambda_1 \) and \( \lambda_2 \) represent \( K_1 \) and \( K_2 \). In order to determine if the ratios are sufficiently small for \( \lambda_1 \) and \( \lambda_2 \) to be considered accurate representations of \( K_1 \) and \( K_2 \) for potassium in the perfused papillary muscle, the following experiment was done: The exchange of potassium and that of a substance, sucrose, whose interstitial exchange could be considered similar to that of potassium were studied simultaneously. Since sucrose remains predominantly extracellular all the ratios above approach zero. A papillary muscle was labeled simultaneously with \( K^{12} \) and sucrose-\( C^{14} \) and subsequently washed out. Effluent plots were then obtained both for \( K^{12} \) and for sucrose-\( C^{14} \) by analyzing samples immediately for total activity and then once again six days later at which time only 0.03% \( K^{12} \) activity remained. The values of the later analysis were then subtracted from the earlier plot to give the simultaneous \( K^{12} \) and sucrose-\( C^{14} \) washouts. Sucrose washed out with a rate constant of 0.53/min and potassium with a phase 1 rate constant of 0.63/min.

Exchange studies (in progress) of sodium which has a \( C_iV_i/C_oV_o \) of \( 0.3 \) indicate a \( \lambda_1 = 0.64/\text{min} \). Calcium, which has a more complex kinetic compartmentalization,

\[ \frac{C_iV_i}{C_oV_o} = 20 \]

in the perfused papillary muscle. This indicates that \( K_1 > K_2 \) for potassium. Therefore, though potassium may be serially arranged in the tissue, its exchange can be considered parallel from a kinetic viewpoint. Consequently, all calculations of exchange constants and membrane fluxes will assume a parallel system to be operative.

EXCHANGE DEPENDENCE ON FIBER DIAMETER

The possibility that phase 3 might arise entirely on the basis of variations in fiber diameter was investigated. The diameters of 100 fibers in a papillary muscle were measured (from formaldehyde-fixed hematoxylin-eosin stained sections). Fibers with centrally placed nuclei were selected to insure against oblique sectioning. These tended to be nearly circular in configuration. Although some shrinkage, which would tend to decrease variation in size, was to be expected in fixation, variation was still considerably greater than that found by Roberts and Wear. The tissue washout curve was selected for a typical muscle and is illustrated in figure 4. In this muscle \( \lambda_2 = 0.0145/\text{min} \) initially and began to deviate from this exponential at 100 minutes. For comparison, a washout curve was constructed for a muscle all of whose fibers were assumed to have a rate constant (\( \lambda \)) of 0.0145/\text{min}, a mean fiber diameter (\( d \)) of 18.9 \( \mu \) and the diameter (\( d_o \)) of its fibers distributed according to the proportions (\( a \)) indicated in table 1.
TABLE 1

<table>
<thead>
<tr>
<th>Fiber diameter (d), µ</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>16</th>
<th>17</th>
<th>18</th>
<th>19</th>
<th>20</th>
<th>21</th>
<th>22</th>
</tr>
</thead>
<tbody>
<tr>
<td>Per cent of total (a)</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td>15</td>
<td>18</td>
<td>16</td>
<td>15</td>
<td>12</td>
<td>8</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Mean diameter, µ</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>16.9</td>
</tr>
</tbody>
</table>

**FIGURE 4**

Semilogarithmic plot of an actual tissue washout (x) compared with a theoretical washout (•) constructed on the basis of fiber size distribution as described in the text. The broken line represents extrapolation for a washout with single exponential characteristics.

Activity (Y) at time (t) relative to the initial count (Y₀) can then be derived from

\[ \frac{Y}{Y₀} = a_1 e^{-(d/d_1) \lambda t} + a_2 e^{-(d/d_2) \lambda t} + \ldots + a_n e^{-(d/d_n) \lambda t} \quad (1) \]

It is apparent that the calculated theoretical curve does not deviate from that defined by a single exponential until washout has continued for 300 minutes. The experimentally defined curve, however, demonstrates that deviation commences before 100 minutes and is marked by 200 minutes. Therefore, a variation in fiber diameter alone is not adequate to account for the appearance of phase 3.

**K⁺ CONTENT OF PHASES 1 AND 2**

The total K⁺ content represented by a phase was calculated in seven muscles using the relation

\[ K⁺ \text{ content} = \frac{Y₀/\lambda \times 10^3 \text{ ml}}{f \times S₀ \times \text{ wet vol of muscle (ml)}} \quad (2) \]

where \( Y₀ = \) counts/min/min at the instant washout was commenced. This is the intercept value for the phase being calculated.

\[ \lambda = \text{rate constant (min}^{-1}\text{)} \text{ for phase, defined graphically.} \]

\[ f = \text{fraction of phase labeled immediately prior to washout assuming equivalent uptake and washout rate constants} = 1 - e^{-\lambda t} \]

\[ S₀ = \text{specific activity of the perfusate (counts/min/mmoles K⁺)} \]

The K⁺ content of phase 0 was not calculated from equation 2 because of difficulty in ascertaining its exact intercept value because the first few effluent droplets after commencing washout contain some loading solution from the perfusion system.

Analysis of the seven muscles, perfused for an average 202 minutes, indicated a mean value of 2.06 mmoles K⁺/liter tissue water for phase 1 and 39.3 mmoles K⁺/liter tissue water for phase 2.

**K⁺ WASHOUT AT VARYING PERFUSATE CONCENTRATIONS OF K⁺**

The effects produced on the exchange characteristics of potassium by altering K⁺ concentration of the perfusate are illustrated in figure 5. Simultaneous direct tissue and effluent counts are plotted semilogarithmically during washout of K⁺ in a muscle which had been labeled for 60 minutes with perfusate containing K⁺ in concentration of 4 mM. Washout during the first 20 min with 4 mM K⁺ had established a \( \lambda_2 \) of 0.0116.
The standard perfusate was then replaced by one containing 16 mM K⁺. The activity of K¹⁸ in the effluent increased almost twofold immediately, declined rapidly, and then rose again to establish, finally, a slope very nearly parallel to that obtained from the total tissue at λ₂ of 0.0266 min⁻¹. The altered rate constant remained stable throughout the course of the 16 mM K⁺ perfusion, but then returned toward control value when perfusion of 4 mM K⁺ was restored at 43 minutes.

In a second muscle the exchange characteristics were altered in a similar way by 16 mM K⁺ from 0.0133 to 0.0230 min⁻¹. Perfusion with 0 mM K⁺ decreased λ₂ by approximately 50% in two muscles.

It should be noted in figure 5 that there was a 12-minute delay before the characteristics of altered exchange became fully manifest in the effluent following the change in K⁺ concentration of the perfusate.

**K⁺ Exchange and Increased Frequency of Contraction**

Figure 6A illustrates portions of semilogarithmic tissue and effluent plots from a papillary muscle in which the frequency of contraction was increased from 18 to 38 beats/min at the 30th minute of washout and continued until the 50th minute when a rate of 18/min was re-established. The mechanical parameters of systolic and diastolic tension are indicated for various periods of the isotropic washout. The increment in frequency of 20 beats/min was not accompanied by either a positive or negative tension staircase.
The systolic and diastolic tensions remained stable throughout the experiment except for a small (0.1 g) transient increase in diastolic tension for the first two minutes of the period of augmented frequency. The effluent activity increased transiently for two minutes, fell and then increased again to remain above the extrapolated control effluent plot for the next 16 to 17 minutes or until approximately two minutes prior to return to a frequency of 18 beats/min. The maximum increment in effluent activity was reached at eight minutes following the onset of increased frequency of contraction. It should be noted that there was no detectable alteration in the slope of the tissue plot coincident with the period of augmented frequency. This indicates that the exchange constant representing phase 2 was not affected significantly by the change in frequency of contraction. The transient increase in effluent activity represents therefore a small net loss of K+ from the region of the muscle represented by phase 2 (see Discussion).

The total increment in effluent K\textsuperscript{42} activity between the 32nd and 48th minute of washout was calculated by summing the differences in counts between each droplet and the extrapolated effluent plot for this period. The total net K\textsuperscript{+} loss can then be calculated from the relation:

\[
K^+ \text{ (mmoles/liter)} = \frac{\sum \Delta \text{cpm} \times 10^3 \text{ ml}}{S_m \times \text{wet vol of muscle (ml)}}
\]  

(3)

where \(\sum \Delta \text{cpm}\) = total difference in counts for the period

\(S_m\) = specific activity of phase 2 portion of the muscle at start of augmented frequency period \(= (1 - e^{-\lambda t_1}) (1 - e^{-\lambda t_2})S_0\)

where \(t_1 =\) labeling period, \(t_2 =\) washout period to start of increased frequency of contraction and \(S_0\) = specific activity of perfusate (cpm/mmolar K\textsuperscript{+}).

The net K\textsuperscript{+} loss associated with augmented frequency in the muscle illustrated in figure 6A was 0.26 m mole/liter tissue H\textsubscript{2}O or 0.7 \(\mu\)mole/liter tissue H\textsubscript{2}O/beat.

Figure 6B represents the same type of experiment as illustrated in figure 6A, but in this muscle the mechanical responses to increased frequency of contraction were markedly different. An increment in frequency of 30 beats/min resulted in a positive tension staircase (+52\%) and then a progressive decline.
in systolic tension to 30% of control associated with moderate contracture (1.8 g increase in diastolic tension). On return to lower frequency of contraction recovery was not complete and contracture persisted. K* effluent activity followed the same sequence as illustrated in figure 6A. There was a small initial increase and decline, followed by a greater and more prolonged increment. The maximum increment in effluent activity was reached, as in figure 6A, at approximately eight minutes but the curve remained elevated throughout the period of augmented frequency and for six to seven minutes following return to control frequency. Again, there was no detectable alteration in the slope of the tissue plot coincident with the period of augmented frequency. The tissue and effluent plots were divergent in this experiment, as in figure 3, indicating a relatively larger phase 3.

The net K* loss associated with augmented frequency in the muscle illustrated in figure 6B was 0.81 mmole/liter tissue H2O or 1.4 μmoles/liter tissue H2O/beat. This amounts to 3.1 and 2.0 times the respective values for the muscle represented in 6A although both muscles were perfused rapidly at the same rate of 2.5 ml/g tissue/min.

Table 2 summarizes the data from nine muscles in which frequency of contraction was augmented to varying degrees. It should be noted that the two muscles (F and H) which showed no staircase and stable mechanical function had the smallest net K* losses. The muscle demonstrating the largest staircase (D) lost the most K*. Results from the other muscles in the group are between these two extremes. The mean total net K* loss of 0.93 mmole/liter amounts to 2.4% of the estimated content of the region represented by phase 2. This would not be expected to be evident in the plot of tissue activity although it can be recognized readily and quantified in the more sensitive effluent analysis.

Discussion

KINETICALLY-DEFINED K* PHASES

There is evidence for the existence of at
least four exponentially-defined phases of K⁺ exchange.

Phase 0
The exchange constant for phase 0 (λ₀ = 3.2/min) is similar to that noted for I₁⁺-labeled albumin (λ = 4.1/min) in the perfused papillary muscle. This suggests that this most rapidly exchanging phase is predominantly representative of K⁺ in the vascular compartment. This compartment has been measured previously at 7% of the wet weight of the perfused muscle. At a K⁺ concentration of 4 mM in the perfusate this would account for approximately 0.3 mmole K⁺/liter tissue water. A similar phase 0 was found for Ca⁺.

Phase 1
The exchange constant for phase 1 (λ₁ = 0.65/min) is similar to that noted previously for sucrose (λ = 0.58/min) and for Ca⁺ (λ₁ = 0.59/min). In addition, simultaneous sucrose and K⁺ washout in the same muscle indicated that λ₁ for K⁺ was in the same range as the exchange for sucrose. The study of Page and Bernstein indicates that the interstitial diffusion channel is constant for small molecules of different size and that the dimensions of the diffusion channel are large relative to sucrose and smaller species. These data suggest that λ₁ is predominantly representative of K⁺ in the interstitial space.

The total K⁺ ascribable to phase 1 was 2.06 mmole/liter tissue water. If the interstitial K⁺ concentration equals that of the perfusate (4 mM), then the interstitial K⁺ space represents 51.5% of the total tissue water or 42% of wet weight of tissue perfused for three to four hours.

An approximation of the efflux from the phase 1 compartment can be derived from the relation:

\[ m_e = \lambda_1 C_o V/A \]  

where \( m_e \) = efflux  
\( \lambda_1 \) = exchange constant for phase 1 (assumed to represent interstitial K⁺)  
\( C_o \) = interstitial concentration of K⁺  
\( V \) = volume of compartment  
\( A \) = surface area of compartment

If the surface area of the interstitium is equivalent to the estimated capillary surface area of 1000 cm²/cm³, equation 4 gives a flux of 18.4 μmoles/cm²/sec or 1.10 μmoles/g wet tissue/min.

Phase 2
\( \lambda_2 \) for K⁺ is 0.0139/min as measured by analyses both of tissue and of effluent during washout of K⁺ in muscles which had been perfused for three to four hours and isotopically labeled up to 85 minutes. The total K⁺ ascribable to this phase derived from effluent curves and equation 2 was 39.3 mmole/liter tissue water. If phase 2 represents K⁺ of intracellular origin, then intracellular K⁺ concentration equals 98 mM in papillary muscles perfused for three to four hours.

The efflux from this compartment can be derived from equation 4 if we assume phase 2 represents K⁺ of intracellular origin. A cellular \( V/A \) ratio of 4.23 × 10⁻⁴ cm can be derived using the mean fiber diameter of 16.9 μ (table 1) and assuming a cylindrical cell. Equation 4 then gives a flux of 9.6 μmoles/cm²/sec or 0.46 μmole/g wet tissue/min from this compartment.

The total flux from phases 1 and 2 thus is 1.56 μmoles/g wet tissue/min. At the perfusion rate used (1 ml/g/min) the vascular turnover of K⁺ is 4 μmoles/g wet tissue/min or 2.6 times the calculated total maximum flux. This indicates that tissue K⁺ exchange is not perfusion limited in the perfused papillary muscle.

The total tissue potassium derived from summatting phase 0, 1, and 2 is 41.7 mmole/liter tissue water. Analysis of muscle perfused for three to four hours indicated a content of 41.7 mmole/liter (fig. 1). This indicates that virtually all of the tissue potassium is accounted for in these three kinetically-defined phases.* There is, however, kinetic evidence for an additional phase, phase 3.

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*The quantitative analysis of the K⁺ content of phases 1 and 2, based on the assumption of parallel arrangement of compartments, indicates compartmental and total K⁺ contents which are reasonable and agree with total tissue analysis. This supports the view that K⁺ exchange can be defined realistically in terms of a parallel arrangement of compartments.
Phase 3

Although quantitatively very small (probably less than 1% of total tissue K⁺ in most muscles) this phase gives evidence of its presence particularly in the experiment illustrated in figure 3 (also figure 6B). In the experiment illustrated in figure 3, the papillary muscle was isotopically labeled for over three hours and, when washed out, produced nonparallel tissue and effluent curves. As has been shown by Persoff it gives evidence for the existence of a more slowly exchanging compartment not evident in short-term effluent analyses alone. The origin of this kinetically-defined phase 3 cannot be derived with any assurance.

It should be noted that a large net loss of tissue K⁺ occurred prior to the kinetic studies. The t½ for this loss was approximately 60 minutes. It is possible, though unlikely, that this net K⁺ loss was derived from a separate phase. It is more likely that this K⁺ was lost from phase 2.

POTASSIUM EXCHANGE ASSOCIATED WITH CHANGES IN K⁺ PERFUSATE CONCENTRATION

The tissue exchange rate of K⁺ is markedly affected by alteration in the concentration of K⁺ in the perfusate (fig. 5). Increasing the perfusate concentration fourfold in two experiments produced a 2.3- and a 1.7-fold increase in λ₂. Perfusion with zero K⁺ produced a 50% reduction in λ₂. The results are similar to those of Carmeliet in Purkinje fibers. Though membrane potentials were not measured in the present study, Carmeliet has calculated that the change of potential produced by (K)₀ in the range of 16 mM K⁺ would be sufficient to explain the marked alteration in rate constant without alteration in the potassium permeability of the membrane. This calculation is based on the Goldman constant field equation. It is possible, however, that an increased permeability does contribute to the changes observed. This possibility was suggested by Weidmann in his experiments on turtle ventricle and Carmeliet indicates that the alterations produced by zero K⁺ perfusion must involve a decrease in K⁺ permeability since the change in membrane potential cannot account for the marked decrease in exchange constant.

The alterations in phase 2 K⁺ exchange by changes in perfusate concentration strongly support the assumption that this phase represents intracellular K⁺. It should be noted that an alteration in the overall exchange rate of cellular K⁺ is clearly manifest in both the effluent and tissue curves (in contrast to the rate-induced changes in K⁺ flux as indicated below).

The diphase response in effluent isotopic activity is interesting. The initial spike correlates with the rapid onset of a moderate increment in diastolic tension and some contracture following high K⁺ perfusion. Contracture of the muscle would be expected to produce a reduction or “squeezing out” of interstitial volume with attendant transient loss of isotope from the interstitial space. A similar transient was described in effluent Ca²⁺ activity after commencing a contracture-inducing low Na⁺ perfusion. No discernible alteration is found in the tissue curve during the inscription of the initial spike. This indicates that it represents a portion of tissue containing relatively little isotope. This is expected of the interstitium after 20 min of washout.

The 12 min delay before the slope of the effluent curve is fully established following 16 mM K⁺ perfusion is consistent with an interstitial exchange constant (λ₁) of 0.65/min. The interstitium has to equilibrate to the high K⁺ concentration before its full effect can be registered at the cell membrane. Then the interstitium has to equilibrate again to the increased flux from the cell before a stable slope can be established for the effluent activity. Two “complete equilibrations” (98%) of phase 1 or interstitium would theoretically require 12.0 minutes.

POTASSIUM EXCHANGE ASSOCIATED WITH INCREASED FREQUENCY OF CONTRACTION

(a) Cellular Exchange

Increments in frequency of contraction from control rates as low as 1/min to as much as 38/min failed to alter the slope of the tissue plot during isotopic washout in a total of 12 muscles (figs. 6A and B). This indicates that
the overall exchangeability of cellular potassium is not influenced by rate of contraction in these studies. The present results are in accord with those of Schreiber et al. and Brady (unpublished observations quoted in Brady and Woodbury) in guinea pig heart and frog heart, respectively. On the other hand, studies of Wood and Conn, Conn and Robertson, Humphrey and Johnson, Rayner and Weatherall, and Persoff all indicate an increase in K⁺ exchange associated with increased frequency of contraction.

The present results in the dog papillary muscle are consistent with the theory suggested by Brady and Woodbury and by Noble. If it is granted that efflux of K⁺ ion is largely responsible for the rapid repolarization associated with phase 3 of the action potential, then an increase in K⁺ efflux above basal levels during this phase would have to be largely cancelled by a decrease in K⁺ efflux below basal levels during another portion of the cardiac cycle.

The most likely period for a decrease in potassium conductance (proportional to permeability) to occur is during the plateau (phase 2) of the action potential. Electrophysiological evidence for this has been found in cardiac muscle by Weidmann (kid Purkinje fibers), Hall et al. (dog and sheep Purkinje fibers), Johnson et al. (rabbit ventricle), Johnson and Wilson (cat, dog, sheep, rat, guinea pig ventricle), and Lorber et al. (frog ventricle). The present study is consistent with the concept of a significant reduction of potassium conductance below resting levels during the course of the action potential in mammalian ventricular muscle.

(b) Transient Net Loss

The transient increase in effluent activity, during increase in frequency of contraction (figs. 6A and 6B), at a time when the overall exchangeability was unaltered indicates a transient net loss of a small fraction of cellular K⁺. If the effluent curve represented the tissue turnover of K⁺ as a whole, the tissue curve would alter and become parallel to the effluent curve after it had reached its peak of activity at approximately 8 min. This was noted in figure 5 during high K⁺ perfusion. The tissue curve remained unaltered in 12 muscles following increases in frequency of contraction.

The summary of data in table 2 indicates that even the smallest rate increments were associated with a transient intracellular K⁺ loss. The greater the increment in frequency, the larger the K⁺ loss tended to be. The larger K⁺ losses tended to be associated with the greater positive tension staircases with some exceptions. The muscles which progressed into contracture continued to demonstrate net K⁺ losses whereas those with stable mechanical function ceased to lose K⁺.

The mean K⁺ loss of 2.4% from phase 2 (assumed to be intracellular) is near the value found by Hajdu to be associated with maximal increments in tension in frog ventricle. Hajdu states that contracture did not appear until more than 7% of internal K⁺ was lost. The present study indicates that contracture may appear in mammalian ventricular muscle at a time when net K⁺ loss is much less (table 2). This may be due, in part, to the large net loss of K⁺ which had occurred in the perfused tissue prior to the increase in frequency of contraction. Sarnoff et al. has also demonstrated a net loss of K⁺ which persisted for the duration of brief (2 min) periods of increased rate induced in the blood-perfused isolated dog heart at 37°C. These losses, if representative of periods of longer stimulation, would approximate those found in the present study.

The time course of the K⁺ loss is particularly interesting. It can be noted in both figures 6A and 6B that increased frequency of contraction produced a small transient increment in effluent activity lasting approximately 2 min. This preceded the more prolonged transient discussed above and could be demonstrated consistently. Since there is a small increase in diastolic tension with increases in frequency, the initial increase in effluent activity probably results from “squeezing” the interstitial volume as was suggested to explain the larger effluent activity spike which accom-
panied perfusion with solution containing 16 mm K⁺.

Of greater importance is the more prolonged transient which attained its peak value at a mean 8.6 minutes (table 2). The source of the K⁺ which is represented by this transient is almost certainly intracellular. If increase in frequency of contraction induced the maximal increment in K⁺ efflux immediately (a step-function) it might be expected that its full manifestation in effluent would be delayed at the cell membrane only by the time required for interstitial equilibration. Since the mean exchange constant of phase 1 (interstitium) is 0.65 min⁻¹, 95% interstitial equilibration would be expected within 4.5 minutes. All muscles except one (A in table 2) showed significantly longer periods to attain their peak transient values. This indicates that the process which determines the transient K⁺ loss requires time to develop and, on the basis of its consistently prolonged downward slope (figs. 6A and 6B), even more time to subside. If the time required for interstitial equilibration is considered, it would seem that the rate of intracellular K⁺ loss does not begin to decrease for approximately four to five minutes and then requires approximately twice as much time to cease. These time parameters are for those muscles in which increments of frequency are maintained for 20 minutes and in which active tension remains stable without contracture (table 2, muscles F and H). K⁺ loss continues in the presence of progressive contracture (muscles G and I) or ceases within two to three minutes (corrected for interstitial equilibration) after cessation of shorter (ten to fifteen min) periods of stimulation (muscles A to E).

CORRELATION OF K⁺ AND Ca²⁺ WITH INCREASED FREQUENCY OF CONTRACTION

Previous studies¹⁻³ of Ca²⁺ exchange in the dog papillary muscle indicated a net increase in myocardial Ca²⁺ associated with increments in frequency of contraction. This net gain in Ca²⁺ was reversed in the face of continued increment in rate, if the rate increase did not result in progressive fall of contractile tension and onset of contracture. The maximum increment in tissue Ca²⁺ was reached after ten to twelve minutes, represented a mean 2.5 μmoles/liter/beat and could be kinetically-localized to the phase 2 portion of Ca²⁺ exchange (λ₂ = 0.116/min).

The average time course of the rate of K⁺ loss in the present study is related to the course of net Ca²⁺ movement in previous studies in figure 7. The curves are uncorrected for interstitial equilibration time since the phase 1 (interstitial) rate constants are very nearly equal for K⁺ and Ca²⁺. It can be seen that net uptake of Ca²⁺ continues while the rate of K⁺ loss is increasing. Three to four minutes after the onset of decline in the rate of K⁺ loss, net Ca²⁺ movements reverse (the peak increment represents approximately 10% gain in tissue Ca²⁺) and tissue Ca²⁺ returns to control levels. The curves represent the patterns of change in muscles without progressive contracture. In muscles which demonstrate deterioration of function, with progressive contracture, K⁺ loss and Ca²⁺ gain continue. The larger increments in frequency of contraction are commonly associated with more rapid and larger K⁺ losses and with greater net gains in tissue Ca.³ These muscles also commonly demonstrate large initial tension staircases.

It is probable that the K⁺ movements are evidence of Na⁺ ion shifts within the myocardium. It has been postulated² that, due to a lag in the ability of the Na⁺ pump to
compensate immediately for an increase in frequency of contraction, there is a transient displacement of Na\(^+\) intracellularly from a membrane region (sarcoplasmic reticulum) where Na\(^+\) and Ca\(^{2+}\) are in competition for sites. The course of net K\(^+\) loss demonstrated by this study is consistent with, but by no means proof of, such a transient displacement.

If the K\(^+\) loss represents a "Na\(^+\) pump lag" an estimate of this lag can be made. Total mean net K\(^+\) loss was 0.93 mmoles/liter tissue H\(_2\)O for a 16-min period of a 26.6 beat/min increment in rate (table 2). The active Na\(^+\) flux/beat is estimated from the relation \(M = CV/F\) where \(M\) is the molar concentration of Na\(^+\), \(C\) is the membrane capacitance, \(V\) is the voltage change, and \(F\) is the Faraday. \(C\) is assumed\(^{33}\) to be 11 \(\mu\text{F/cm}^2\) and \(V = 110\) mv for mammalian ventricular muscle. The unidirectional Na\(^+\) flux associated with each stimulus would be 12.5 \(\mu\text{mole/cm}^2\) or 12.7 mmoles/liter tissue H\(_2\)O. A 16-minute period of a 26.6 beats/min rate increment would, therefore, increase Na\(^+\) flux by 5.4 mmoles/liter tissue H\(_2\)O. If the 0.93 mmolar K\(^+\) loss represents intracellular Na\(^+\) accumulation, then the average "lag" in Na\(^+\) pump activity over the 16 minutes would be 17%.

If the postulated Na\(^+\) vs. Ca\(^{2+}\) competition is on the basis of electrical charge (only one of several possibilities) then one would predict an approximate maximal increment of 0.47 mmoles Ca\(^{2+}\) for a 27 beats/min increment in rate. Analysis of 10 muscles\(^3\) in which an average 27 beats/min increment was introduced showed a mean maximal increment of 0.63 mmoles Ca/liter tissue H\(_2\)O.

**Summary**

Four phases (0 to 3) were defined kinetically for potassium in the dog papillary muscle when perfused arterially for four to five hours with solution containing 4 mM/liter K\(^+\). Total tissue K\(^+\) fell from 91.1 ± 1.99 to 41.0 ± 1.06 mmoles/liter after three hours perfusion. It then remained stable during the period when kinetic studies were done. The mean rate constant \(\lambda\) (min\(^{-1}\)), potassium content (mmoles/liter tissue water) and suggested origin of each phase are respectively: (phase 0) \(\lambda_0 = 3.2, 0.3\), vascular; (phase 1) \(\lambda_1 = 0.65, 2.06\), interstitial; (phase 2) \(\lambda_2 = 0.0139, 39.3\), intracellular; (phase 3) \(\lambda_3 < 0.004, < 0.5\), origin unknown.

Alteration of K\(^+\) concentration in perfusing fluid produced significant changes in the intracellular exchange rate of K\(^+\). This was in marked contrast to increments in frequency of contraction which had no effect on the overall exchangeability of intracellular potassium. Increases in rate, however, were associated with a transient net loss of intracellular K\(^+\). This loss continued if the active tension of the muscle declined and if contracture progressed. The loss ceased if muscle function remained stable during continued increased frequency of contraction. A positive tension staircase was approximately proportional to the net K\(^+\) loss. The net K\(^+\) loss was 0.93 mmmol/liter tissue water in nine muscles in which a mean 27 beats/min rate increment was introduced for a mean of 16 minutes. This represented 2.4% of intracellular K\(^+\).

A significant time lag was found before the net K\(^+\) loss reached a maximum rate and began to decline. This is compared with the previously demonstrated transient net increment in Ca\(^{2+}\) uptake that accompanies increased frequency of contraction. These ionic movements are consistent with the theory that Na\(^+\) movements in and out of a "specialized membrane region" are related to Ca\(^{2+}\) movements and thereby influence the control of myocardial contractility.

**References**

Potassium in Dog Ventricular Muscle: Kinetic Studies of Distribution and Effects of Varying Frequency of Contraction and Potassium Concentration of Perfusate

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