Kinetics of Rubidium Uptake in the Working Dog Heart

By Wilfred H. Ziegler and Carl A. Goresky

ABSTRACT

More than 50% of tracer rubidium entering the coronary circulation is actively taken up by the heart muscle cells during a single passage. Rubidium ions must traverse the capillary wall, the interstitial space, and the muscle cell membrane to enter the very large potential rubidium pool within the cells. The aim of this investigation was to determine which, if any, of these steps are rate limiting. The anterior descending branch of the left common coronary artery was perfused at its origin. Pulse injections containing $^{86}\text{Rb}^+$, $^{22}\text{Na}^+$ or $^{14}\text{C}$-sucrose, and $^{125}\text{I}$-albumin were made into the perfusion line and timed serial samples of coronary sinus blood were collected and analyzed. A model was designed which incorporates rate constants describing both the exchange of $^{86}\text{Rb}^+$ at the capillary wall and its entry at the muscle cell membrane. Labeled sucrose or $^{22}\text{Na}^+$ was used to determine flow per interstitial fluid volume, a parameter necessary for the application of the model. It was assumed that the interstitial fluid volume available for labeled sucrose or $^{22}\text{Na}^+$ was identical to that available for $^{86}\text{Rb}^+$. The rate constant describing exchange at the capillary wall (the permeability surface product per unit accessible interstitial fluid volume) increased with perfusion, whereas that for uptake by the myocardial cells was relatively constant and independent of flow.

KEY WORDS indicators model analysis capillaries coronary microcirculation working heart $^{86}\text{Rb}^+$ cardiac muscle membrane transport

At normal perfusion rates the heart actively removes more than 50% of labeled rubidium or potassium entering the coronary circulation. Both of these tracer ions are concentrated in cardiac muscle cells, and during the uptake process, traverse a multi-component barrier consisting of capillary wall, interstitial fluid compartment, and sarcoplasmic membrane. Adrian (1) has shown that the effects of rubidium and potassium on muscle tissue are similar; and that rubidium can substitute to a large extent for potassium in living muscle cells. Indeed, 72 hours after infusion of $^{86}\text{Rb}$, the ratio of left ventricular to plasma activity averages 1.06 times the corresponding concentration ratio for unlabeled potassium (2). The potential intracellular rubidium pool is therefore of approximately the same size as the potassium pool.

The purpose of the present investigation is to quantify the manner in which the components of the barrier limit the exchange of labeled rubidium between the intracapillary and intracellular compartments. The problem has been approached in two ways in previous investigations.

1. Lymphatic outflow concentrations have been considered to be an index of interstitial fluid concentrations. Downey and Kirk (3, 4) showed that the specific activities of $^{42}\text{K}^+$ in coronary lymph approach and become identical to those in coronary sinus blood 15 minutes after the beginning of an infusion in which arterial specific activities are maintained constant. This period is far too short to permit equilibration between intracellular and extra-
cellular $^{42}$K$^+$, and these authors therefore considered that the relative effect of the barrier to diffusion at the capillary wall is small compared to that at the cardiac muscle cell membrane, i.e., that the data could be interpreted to mean that equilibration occurs between extracellular space and blood relatively quickly. Adrian (1) has demonstrated that rubidium uptake into isolated muscle cells is slightly slower than that of potassium, and therefore the myocardial cellular uptake process would be expected to be even more rate limiting in the distribution of labeled rubidium.

2. Outflow indicator dilution curves have been obtained. Three differing kinds of interpretation of the experimental data have been offered. Renkin and Sheehan (5) introduced $^{42}$K$^+$ and $^{86}$Rb$^+$ together with labeled albumin into the fluid perfusing an isolated gracilis muscle. The fraction of labeled rubidium and potassium lost from the first few outflow samples (the transient response) was identical; and the fraction of rubidium lost from later samples (the steady-state response) was comparatively smaller. Since the diffusion coefficients of potassium and rubidium differ by less than 2%, the authors concluded that the early extraction values reflected passive loss limited by a resistance at the capillary wall, with a large sink beyond. The steady-state extraction was taken to be the result of diffusion across the combination of capillary wall, interstitial fluid space, and muscle cell membrane. Renkin and Sheehan considered these three components as resistances in series and inferred, after consideration of the extraction values of serial samples, that approximately two-thirds of the total resistance to $^{86}$Rb$^+$ distribution in this preparation was at the cell wall and that most of the remaining resistance was located at the capillary wall. Much earlier, Conn and Robertson (6) had examined the approach of coronary sinus $^{42}$K$^+$ specific activity to that of arterial blood when the latter was held constant for 25 minutes. To analyze their results, they designed a two-compartment model in which the capillary walls offered no resistance to exchange and in which rapid and complete mixing within both the entire extracellular and intracellular compartments was assumed. The limiting barrier to exchange was placed at the cell walls, and rates of exchange between interstitial and intramyocardial $^{42}$K$^+$ pools were estimated. Friedman (7, 8) examined the inverse relation of $^{86}$Rb$^+$ extraction to blood flow in skeletal muscle. To account for this, he proposed a system consisting of nutritive and shunt pathways in parallel. It was assumed that flow-limited exchange of $^{86}$Rb$^+$ with all compartments (i.e., potentially complete extraction) occurred in the nutritive pathways and that no exchange occurred along the shunt pathways.

In the present study, the multiple indicator dilution method was used to examine the exchange of $^{86}$Rb$^+$, in conjunction with a more detailed model of the process than has hitherto been used. Analysis of the data by use of this model indicates that both the capillary wall and the cardiac muscle membrane act as barriers to the transport of $^{86}$Rb$^+$; that the rate constant at the capillary wall varies with perfusion; and that the rate constant for the uptake of $^{86}$Rb$^+$ by myocardial cells is relatively independent of flow.

**Methods**

In these experiments the anterior descending coronary artery was perfused at its origin, as previously described (9). A sampling catheter was placed in the coronary sinus and the chest was closed. An air cushion was used to damp out the pressure transients resulting from the sudden injection of materials into the inflow line (9).

The dilution experiments were carried out in the following manner. A mixture containing $^{125}$I-albumin (a vascular reference substance), $^{22}$Na$^+$, or $^{14}$C-sucrose (interstitial space reference substances), and $^{86}$Rb$^+$ was introduced as a pulse injection into the perfusion line. Timed serial samples of blood were obtained from the coronary sinus and were assayed for the activities of the materials injected. These, in turn, were expressed as an outflow fraction of the total amount of material injected per milliliter of blood.

**Theory and Results**

**AN ANALOGUE MODEL**

The historical aspects of this problem will best be served by developing first an electrical

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analogue model of the multicomponent diffusion barrier. If the individual compartments are assumed to be well mixed, the single unit shown in Figure 1A results. If the system is considered to be a distributed one, a large number of such units are required in sequence along the length of the capillary, so that the required time delay occurs in the system. Within the single unit, Renkin and Sheehan (5) neglected the capacitances of the interstitial space (C₁ to Cₙ), and therefore return of indicator from the interstitial space was impossible, in their modeling. The resistances R₁ to Rₙ could then be combined into a single resistance for the interstitial compartment. The following evidence, however, shows that R₁ to Rₙ may be neglected and C₁ to Cₙ should be retained. Studies of the distribution of tritiated water and antipyrine in the heart (9) have shown that molecules even the size of antipyrine equilibrate so rapidly that the process is limited solely by flow. The region of the interstitial fluid compartment supplied by an individual capillary has the same maximum dimensions as the total water compartment and one would therefore expect that equilibration of substances within the interstitial compartment would occur just as rapidly. It is therefore unlikely that the interstitial fluid compartment in the heart will offer a measurable resistance to diffusion in a direction normal to the capillary.

FORM OF THE DATA

The data resulting from single rapid injections of a mixture containing ¹²⁵I-albumin, ¹⁴C-sucrose, and ⁸⁶Rb⁺, at two perfusion rates are given in Table 1 and plotted in Figure 2. In both panels of this figure, the initial parts of the labeled sucrose curve are grossly reduced in relation to that for labeled albumin, as a consequence of the movement of the sucrose out into the interstitial space. Later the labeled sucrose curve crosses the labeled albumin curve, and exhibits a prolonged tailing. At this time the material is returning from the interstitial space (9). These experimental curves demonstrate the storage of labeled sucrose in the interstitial space, and so, in the analogue electrical model, C₁ to Cₙ should be retained. The area under the early part of the ⁸⁶Rb⁺ curve is greatly reduced in relation to that for labeled sucrose, in each instance, and indicates uptake of material from the interstitial space.

The volumes of the spaces accessible in both the capillary wall and the cell membrane of the muscle cell are extremely small, and therefore C_c and C_cm should be neglected. In the muscle cell, the potential ⁸⁶Rb⁺ pool is extremely large. Sheehan (10) estimated that at equilibrium there would be about 200 times as much ⁸⁶Rb⁺ inside skeletal muscle cells as elsewhere in the muscle tissue. Renkin (11) found that the ⁴²K⁺ extraction fell by only 7% over a period of 40 minutes in gracilis muscle. Therefore, by analogy, return of ⁸⁶Rb⁺ from the intracellular compartment during a 30-second pulse experiment must be extremely small. Hence capacitance C_m can be consid-
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Data from Experiment Underlying Figure 2

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</table>

*Run 1 of the first experiment listed in Table 2.
†Run 3 of first experiment listed in Table 2.

The next step is to translate this electrical model into a single capillary model, which in turn may be used as a building block for a whole-organ model. In a preceding paper (9), we considered heart muscle to consist of identical average Krogh cylinders in which longitudinal diffusion is neglected and radial equilibration within compartments is assumed to be instantaneous. An extension of this model, to take into account cellular uptake, can be derived as follows:

**Conservation of Matter**

\[
\begin{align*}
\left( \frac{\Delta x}{A} \right) \frac{\partial u}{\partial t} &= -Q \frac{\partial u}{\partial x} \Delta x - \left( B \Delta x \right) \frac{\partial v}{\partial t} - \left( B \Delta x E v \right) \\
\end{align*}
\]

(1)

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Outflow patterns of $^{131}$-albumin, $^{14}$C-sucrose, and $^{86}$Rb$^+$. These patterns correspond to runs 1 and 3 of a group of four obtained on the same dog. The derived parameter $F$, the flow per unit accessible interstitial volume, was found to have the value 0.16 sec$^{-1}$, for the set of data on the left; and the value 0.087 sec$^{-1}$, for the set on the right.

Fick's Law of Diffusion

\[
\left( \frac{B \Delta x}{A} \right) \frac{\partial v}{\partial t} = \left[ -P \frac{\Delta x}{L} (v - u) \right] - \left[ R \Delta x E v \right] \ldots (2)
\]

where

- $A$ = the cross sectional area of the capillary;
- $B$ = the cross sectional area of the interstitial compartment;
- $u(x, t)$ = the concentration in the capillary, at a distance along the length $x$, and at a time $t$;
- $v(x, t)$ = the concentration in the interstitial space, at a distance along the length $x$, and at a time $t$;
- $P$ = the permeability constant of the capillary wall (cm/sec), for the diffusing substance;
- $S \Delta x / L$ = the surface area of the element of the capillary wall subserving exchange;
- $\Delta x$ = the length of an arbitrary small segment of the capillary and Krogh cylinder of length $L$;
- $Q$ = the flow rate in the capillary;
- $t_r$ = the capillary transit time of the reference indicator;
- $E$ = the rate constant for entry of rubidium into the muscle cell (this is considered to be a one-way process because of the very large intracellular sink and the short times over which the data are obtained);
- $K = PS/BL$, the permeability surface product per accessible volume of interstitial fluid (units sec$^{-1}$); and

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\[ F = Q/BL, \text{ the flow per accessible volume of interstitial fluid (units sec}^{-1}). \]

Equations 1 and 2 must then be solved simultaneously, using as boundary conditions

\[ v(x, 0) = 0; \]
\[ u(x, 0) = 0; \text{ and} \]
\[ u(0, t) = \delta(t - \epsilon), \]

where \( \delta \) is an impulse function and \( \epsilon \) is an infinitesimal time interval.

The outflow profile (i.e., the concentration profile emerging from the end of the capillary is

\[ u(t - t_0, K, E, F) = e^{-\frac{K}{F}}(t - t_0) + T(t - t_0, K, E, F), \]

where

\[ T(t - t_0, K, E, F) = e^{-\frac{K}{F} - (K + B)(t - t_0)} \sum_{n=1}^{\infty} \frac{(t - t_0)^{n-1} \left( \frac{K^2}{F} \right)^n}{n! (n-1)!}. \]

The major steps in obtaining this solution are shown in the Appendix. Like that for the model which was explored previously (9, 12), this solution consists of two components. The first component represents material which did not leave the capillary, the throughput material. Since the parameter \( E \) does not appear in this first component, it is evident that the throughput material is not affected by the presence of the intracellular compartment.

The second component, referred to as the tail function or \( T(t - t_0, K, E, F) \), describes indicator which has left the capillary at least once in its travel from the arterial to the venous end, yet was not taken up by the heart cells. Equation 3 reduces to the solution for the case where there is no cellular removal (9), when \( E \) is zero. The addition of the one-way cellular uptake process to the modeling has resulted in the introduction of an additional factor, \( \exp(-E[t - t_c]) \), into the tail function.

Effect of Changes in \( E \).—Figure 3 shows the effect of change in the values of \( E \) on the form of the outflow profile. All of the parameters used are adjusted so they lie in the physiologically encountered range. For these values of the parameters, the tail function closely approximates a diminishing exponential function. Increase in the value of \( E \) produces a more rapid rate of decrease in this function. It should be reemphasized that over the time of observation which we have used, no return of \( ^{85}\text{Rb}^+ \) from the muscle cells is expected, and the process corresponding to return of intracellular label has therefore not been incorporated in the modeling. The carrier process at the cell membrane of the muscle cells which subserves \( ^{85}\text{Rb}^+ \) transport is a highly concentrative one, and the return of label in this kind of process will be expected to produce an exceedingly prolonged tailing, low in magnitude (13). This component of the outflow profile is missing from both our present data and our present modeling. Despite our neglect of it, it is evident in the data accumulated over long times (i.e., that of Conn and Robertson [6], and that of Renkin [14]).

Obtaining the Parameters \( K \) and \( E \) for \( ^{85}\text{Rb}^+ \).—By using the assumptions we used previously (that the ventricle is homogeneous, that the capillary transit times are identical throughout, and that, in the cases we are considering, the interaction between capillaries produces no quantitatively important changes in the predicted outflow profiles), we may extend the present model into a whole-organ model (9). For the interstitial space reference substances (i.e., when \( E \) is zero), families of outflow curves may be generated by use of Eq. 3 and of the \( ^{125}\text{I}-\text{albumin} \) curve, and the least squares process may be used to arrive at those values of the parameters \( K \) and \( F \) which provide an optimum description of
FIGURE 3

Model outflow profiles for a diffusible indicator undergoing one-way uptake at a cell membrane beyond the interstitial space. The parameter values used to calculate this representative set of profiles were: K = 0.15 sec⁻¹; F = 0.15 sec⁻¹; t_c = 1 second (the corollary of this is that the ratio of the accessible interstitial space to the accessible capillary space, both expressed in terms of ml of plasma, is 6.67); and the series of values, E = 0.0, 0.15, and 0.30 sec⁻¹. The ordinate values have been adjusted so that the normalized area under the whole curve, on a linear plot, when E = 0.0, would be unity. In this illustration the ordinate scale is logarithmic. The first part of the illustrated output is, in each instance (no matter what value is selected for the parameter E), an impulse function with the normalized area, exp(-K/F). It is difficult to illustrate this form, which theoretically has an infinitely large magnitude and an infinitesimally short duration. We have simply placed a vertical line at the time at which the function emerges. The second part of the outflow profile, the tail function, represents returning material. When E = 0.0, all of the exchanging material emerges at the outflow. For the two higher values of E, corresponding values of the tail function are reduced by the factor exp(–E(t – t_c)), and there is a resultant decrease in the underlying area. The area values tabulated on the illustration correspond to those for a linear plot.

the experimental curves for these substances (9).

Now assume that the interstitial space immediately accessible to the extracellular reference substances, ¹⁴C-sucrose and ¹⁴Na⁺, is identical to the interstitial space immediately accessible to ⁸⁶Rb⁺. Then the value for the parameter F, the plasma flow per unit accessible extravascular space (expressed as an equivalent plasma space), will be identical for both the extracellular reference substance

and ⁸⁶Rb⁺. Given a fixed value for F, we may now utilize Eq. 3 in our organ modeling to obtain, by a least squares fit to the experimental ⁸⁶Rb⁺ outflow curves, optimum estimates of the values of E and K for ⁸⁶Rb⁺.

Changes in the Components of the ⁸⁶Rb⁺ Outflow Curves, and in the Parameters K and E, with Flow.—Figure 4 displays the results of least squares curve fits to the ⁸⁶Rb⁺ outflow profiles for the two experimental runs in Figure 2. The calculated curves superimpose fairly reasonably upon the data, apart from the initial samples (this point was amplified in our previous study [9]). Since the return of ⁸⁶Rb⁺ from cells was not included in the modeling, the curve fitting was arbitrarily terminated in the manner shown in the illustration. The calculated curves, in each instance, have been resolved into their two components, throughput and returning material. For the run with the higher flow (that on the left), the returning component is comparable in magnitude to the throughput component. For that with the lower flow (that on the right), the returning component is decreased in magnitude, in relation to the throughput. With the longer time of transit, at the lower flow, a comparatively greater proportion of the exchanging material has been taken up at the muscle cell surface. The reader is to be cautioned against comparing Figures 4 and 3. Although we have assumed that the initial extracellular spaces of distribution of sucrose and Rb⁺ are identical and that the parameter F (the flow per volume of immediately accessible extravascular space) is therefore the same for both of these indicators, the capillary permeability for these two substances is obviously very different. The downslopes of the sucrose and ⁸⁶Rb⁺ curves therefore cannot be compared to Figure 3.

For the experimental run illustrated in Fig. 4A (with an F value of 0.16 sec⁻¹), the corresponding K and E values for ⁸⁶Rb⁺ were 0.264 and 0.224 sec⁻¹; and the K value for ¹⁴C-sucrose, the interstitial space reference, was 0.121 sec⁻¹. For the run displayed on the right (when the perfusion was approximately halved and the F value decreased to 0.057 CIRCULATION RESEARCH, VOL. XXIX, AUGUST 1971
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Experimental and calculated $^{86}$Rb+ curves for the runs illustrated in Figure 2. The ordinate on this illustration is logarithmic. In each instance the curves have been resolved into throughput (the first component), and exchanging indicator (the second component). The latter corresponds to that indicator which has left the vascular space, which has not been taken up by the muscle cells, and which has then returned to the vascular space. It should be noted that the calculated first component of the $^{86}$Rb+ curve, derived by use of the present modeling, is identical in shape to that of the vascular reference curve.

sec$^{-1}$), the values for $K$ and $E$ for $^{86}$Rb+ changed to 0.133 and 0.232 sec$^{-1}$; and the $K$ value for sucrose decreased to 0.076 sec$^{-1}$. For $^{86}$Rb+ the value for $K$ decreased markedly with decrease in flow, whereas that for $E$ hardly changed at all. The pattern of change in these parameters with flow, encountered in the analysis of the above two runs, is a general one. The findings for a number of runs carried out in three dogs are summarized in Figure 5 and Table 2. The figure demonstrates that the parameter $K$ for $^{86}$Rb+, defined as the

<table>
<thead>
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<td><strong>Variation in the Derived Parameters with Flow</strong></td>
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<table>
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<tr>
<th>Interstitial Reference</th>
<th>Run no.</th>
<th>Pump* flow (ml/min)</th>
<th>Hct</th>
<th>$P_t$ (sec$^{-1}$)</th>
<th>$V_{50}$ (ml)</th>
<th>$K_{30}$ (sec$^{-1}$)</th>
<th>$E_{30}$ (sec$^{-1}$)</th>
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*The pump flow is expressed as ml blood sec$^{-1}$.
†The value for $P_t$ is expressed as (ml plasma)/(sec × ml accessible interstitial space). It can be converted to a value for perfusion, expressed as ml min$^{-1}$ g$^{-1}$ if it is multiplied by 5.04 (the product of 60 [sec min$^{-1}$] and the proportion of the ventricle which is immediately accessible interstitial space for sucrose, 0.084 [ml g$^{-1}$]).
‡$V_{50}$ is the interstitial space immediately accessible to the extracellular reference substance.
§$K$ values are permeability surface products per unit interstitial space, expressed as (ml plasma)/(sec × ml accessible interstitial space). These values can be changed to permeability surface products in ml min$^{-1}$ g$^{-1}$ by multiplying by the factor 5.04.
||$E$ may be regarded as a rate constant with the dimensions sec$^{-1}$; or, alternatively, as a unidirectional cellular flux per unit accessible interstitial space. If the latter, its dimensions are converted to ml min$^{-1}$ g$^{-1}$ by the factor 5.04.

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permeability surface product per unit of immediately accessible interstitial space, rises with \( F \) and then tends to level off at higher values of \( F \) (the flow per unit of immediately accessible extravascular space). On the basis of our previous exploration of the behavior of interstitial space reference substances (9), the present pattern is what we would have expected. In contrast, for a given experimental preparation the value of \( E \) remains unchanged as the parameter \( F \) increases.

We have previously interpreted the increase in \( K \) with \( F \) to reflect a recruitment of capillaries with increase in flow, and the relative constancy of the interstitial space with flow to reflect a spatial dispersion of perfused capillaries such that all of the extracellular space remains immediately accessible to the interstitial space reference substances, even at flow rates corresponding to the lower end of the physiological range. The relative constancy of \( E \) with changes in flow implies that the rate of initial sequestration of \(^{86}\text{Rb}^+\) by the muscle fibers is relatively constant and independent of the capillary surface area available for exchange and that all the transport surface of the muscle fibers is accessible to the \(^{86}\text{Rb}^+\), even at lower values of the coronary flow.

**Ratio Curves**—The relation between the curves for the diffusible and vascular reference substances can be emphasized by plotting the ratio \( R(t) \) between the outflow fraction per ml of the diffusible substance and the corresponding values of the intravascular reference substance versus time. In Figure 6 are plotted ratio curves for both \(^{86}\text{Rb}^+\) and its interstitial space reference substance, \(^{14}\text{C-sucrose}\). All four runs from this experiment are included (two of these have been used in previous illustrations). The curves for both \(^{86}\text{Rb}^+\) and labeled sucrose have a similar shape. An initial small decline in the ratio is succeeded by a rapid rise with time. For \(^{86}\text{Rb}^+\), the early values are lower and the rise to values greater than 1.0 is much less rapid. The essential continuity of the data from the various runs, which has been emphasized by Bassingthwaighte et al. (15), is exemplified by these plots. The lowest value of each curve corresponds to the maximum value for the instantaneous extraction, \( E(t) \), since \( E(t) = 1 - R(t) \). The successive runs of the experiment form a continuous sequence. A locus corresponding to minimum \( R(t) \) values (i.e., the maximum extractions) can be defined for both \(^{86}\text{Rb}^+\) and \(^{14}\text{C-sucrose}\).

Yudilevich has proposed that the effect of an uptake process from the interstitial space can be emphasized by simultaneously examining the ratio curves for substances which are taken up at a second barrier and substances for which the second barrier is relatively impermeable (16). No easily interpretable relation between the \(^{14}\text{C-sucrose}\) and \(^{86}\text{Rb}^+\) ratio curves.
curves is seen in the present illustration. However, it is obvious that if the interstitial space reference had a K value identical with that for \( {^{89}}\text{Rb}^+ \), its ratio curve would initially have been identical, and would later have rapidly diverged upward from that of \( {^{89}}\text{Rb}^+ \). Qualitative reasoning, on the basis of ratio curves, will be possible only when the K values for the interstitial reference and the transported substance are either identical or similar in magnitude.

Since we have used a common capillary length in our modeling, we would have expected \( R(t) \) to be a monotonically increasing function of time, for the \( {^{80}}\text{Rb}^+ \) as well as the \( ^{14}\text{C-sucrose} \) (12). We once again would interpret the initially higher values of \( R(t) \) to indicate that there are some short capillaries linked to short nonexchanging channels or that some of the exchanging indicator emerges relatively early, as a consequence of intercapillary interactions. It is also possible that a small amount of diffusional intercommunication occurred between the larger vessels (17). The concentrations of indicator in these early samples are relatively low, and these effects would not be expected to weigh the calculations to any major extent.

**Discussion**

**CHANGES IN THE FORMS OF THE \( {^{89}}\text{Rb}^+ \) CURVES WITH FLOW**

In the present experiments, the changes in the form of the \( {^{89}}\text{Rb}^+ \) curves with flow were interpreted to be consequent to the return of an increasingly higher proportion of the exchanging material, as the perfusion rate was increased. At the higher perfusion rates progressively more capillary surface was mobilized, and the K value increased until it became larger than the E value. A high proportion of the \( {^{89}}\text{Rb}^+ \) continued to exchange but a lesser proportion was sequestered by the sarcoplasmic transport system. Friedman has observed similar changes in the form of \( {^{89}}\text{Rb}^+ \) single passage dilution curves with flow, during a study of \( {^{89}}\text{Rb}^+ \) uptake by isolated perfused skeletal muscle (8). A greater degree of inhomogeneity of perfusion would be expected in this preparation. Despite this, the change in form of the curves appears to have a similar explanation.

**RELATIVE LACK OF CHANGE IN THE PARAMETER E, WITH FLOW**

The main finding in this study was that the rate constant E for the sequestration of tracer \( {^{89}}\text{Rb}^+ \) from the interstitial space into muscle cells is relatively independent of flow. In contrast, the rate constant K, which describes transcapillary exchange and was of the same order of magnitude, increased markedly with flow. The flow dependence of the parameter K for \( {^{89}}\text{Rb}^+ \) was similar to that for tracers which do not enter the intracellular space. In our previous study (9), the size of the interstitial fluid compartment measured by the indicator dilution technique was found to be independent of flow, and the hypothesis was presented that the interstitial compartment could operationally be considered to be well mixed as a result of the random diffusional interconnection between capillaries in this tissue. The implication of the hypothesis is that the total transport surface of the muscle cells will be available to take up \( {^{89}}\text{Rb}^+ \) at all flow rates. The lack of change in E with flow provides substantial support for this hypothesis. It also implies that the spatial distribution of perfusion of the capillaries is such that all fibers in the working heart are easily supplied from perfused capillaries, even when the flow is low. Some of this homogeneity may be subserved by an intermittency of capillary supply, a twinkling phenomenon, particularly at the lower perfusion rates.

**RATIOS OF THE COEFFICIENTS FOR TRANSCAPILLARY EXCHANGE**

In the present experiments, the average value of the ratio of the K for sodium to that for rubidium was 0.66. This is close enough to the ratio of the diffusion coefficients, 0.65, to suggest that the process of transcapillary exchange is passive and that the mechanisms are very much alike for these two ions. However, the ratio \( K_{Na}/K_{Rb} \) decreases regularly and without exception with increase in flow in each experiment and, at the higher flow values, appears to reach not the ratio of free diffusion coefficients but a lower value.
The progression suggests that the ratio may finally approach a ratio of restricted diffusion coefficients. The data must be considered too few for firm conclusions but the observation by Yudilevich et al. (18) of a similar decrease in the ratio of permeability surface product values (sodium: rubidium) with flow in a gracilis muscle preparation suggests that further investigation of the phenomenon is warranted. The average value of the ratio of the K values (sucrose: rubidium) was found to be 0.48. This is somewhat more than the corresponding ratio of their free diffusion coefficients in water, 0.26. The ratio of the K values is once again found to decrease systematically with increase in flow. The ratios of K values considered here will be identical to the ratios of capillary permeabilities for these substances only if our assumption of a common interstitial space is valid. If there is, for instance, a relative degree of steady-state exclusion for sucrose in the interstitial space (19), this will proportionately increase the value of the ratio above that otherwise expected. The degree of exclusion required to make the ratio values for sucrose and sodium consonant is, however, larger than one would expect. Yipintsoi et al. (20) reported observations of ratio values similar to those outlined above and formulated the hypothesis that fixed negative charges in the capillary intercellular clefts produce the relative disparity in permeability to cations and sucrose noted above. The proposal appears to merit more extensive investigation.

LIMITING BARRIERS

The finding of a rate constant for the exchange of 8B Rb+ at the capillary wall of the same order of magnitude as the rate constant for early essentially one-way removal by the myocardial cells implies that both barriers exert major effects on the distribution of this substance within the heart. The size of the potential pools available in the interstitial and intracellular spaces, respectively, are such that relatively early in the time course of the response to steady infusion, the level of activity in the interstitial space would be expected to reach a quasi steady state. Despite this kind of response, one would expect the interstitial fluid activity to remain significantly lower than the plasma activity for that prolonged period of time during which a high net rate of flux of 8B Rb+ or 42K+ from capillaries into muscle cells persists.

Downey and Kirk (3, 4) interpreted their finding that the specific activity of coronary lymph approaches and becomes equal to that in venous plasma within 15 minutes of beginning a steady infusion of 42K+ in light of the assumption that coronary lymph is an index of the average 42K+ interstitial fluid specific activity. If coronary lymph 42K+ were a true measure of average interstitial 42K+ specific activity, the findings would be difficult to reconcile with the existence of a barrier at the capillary wall. The spatial proximity of venous and lymphatic channels raises the possibility of postcapillary equilibration between venous and lymphatic channels. Downey and Kirk discounted this possibility on the basis of the data of Areskog et al. on the isolated heart (21). However, Areskog et al. found that the concentration of potassium in the coronary lymph can exceed that of plasma by as much as 122%, and these findings could be interpreted to be equally incompatible with the assumption of a two-compartment model which neglects the capillary wall as a barrier to the exchange of potassium.

The three-compartment model presented in the present paper, one which assumes a relatively passive barrier at the capillary wall and a concentrative transport process at the muscle cell surface appears to account more adequately for the exchange of 8B Rb+ in the working heart.

Appendix

The solution of the simultaneous Eqs. 1 and 2 is outlined here. Divide each by BΔx:

\[
\frac{A \frac{du}{dt}}{B} = - \frac{Q \frac{du}{dx}}{B} - \frac{dv}{dt} - Ev; \tag{5}
\]

\[
\frac{\partial v}{\partial t} = - K(v - u) - Ev. \tag{6}
\]

Multiply Eq. 5 by B/A and apply the Laplace transformation,
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\[ l \{ f(x, t) \} = \int_0^\infty f(x, t) e^{-\lambda t} dt = \Theta(x, s), \]
to Eqs. 5 and 6:

\[ s\Theta = -\frac{Q}{A} \frac{d\Theta}{dx} + \frac{B}{A} s\Theta - \frac{B}{A} E\theta; \quad (7) \]
\[ \frac{\Theta}{s + K + E}. \quad (8) \]

Substitute Eq. 8 into Eq. 7. Then

\[ \Theta(x, s) = \Theta(0, s) e^{\left(\frac{-sA}{Q} \left[ e^{(s + K + E)} + (s + K + E) \left(\frac{Ax B}{Q A} + \frac{K^2}{s + K + E} + \frac{(Ax B)^2}{(s + K + E)^2} + \cdots \right) \right] \right)}. \quad (9) \]

The last term in the exponent may be eliminated. Then

\[ \Theta(x, s) = \Theta(0, s) e^{\left(\frac{-sAx}{Q} e^{(s + K + E)} - \frac{sAx}{Q} e^{(s + K + E)} \right)} e^{\left(\frac{sAx B}{Q A} + \frac{K^2}{s + K + E} + \frac{(Ax B)^2}{(s + K + E)^2} + \cdots \right)}. \quad (9A) \]

At the outflow from the capillary (i.e., at \( x = L \))

\[ \frac{AL}{Q} = t_e = \text{capillary transit time, and} \]
\[ \frac{B}{A} t_e = \frac{1}{F} = 1/(\text{flow per accessible volume of interstitial fluid}). \]

Whence

\[ \Theta(L, s) = \Theta(0, s) e^{-sAx} e^{-\frac{K^2}{F}} \]
\[ + \Theta(0, s) e^{-sAx} e^{-\frac{K}{F}} \sum_{n=1}^\infty \left(\frac{K^2}{s + K + E} + \frac{(Ax B)^2}{(s + K + E)^2} + \cdots \right) n! . \]

The appropriate inversion into the time domain can now be performed.

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