Capillary, Interstitial, and Cell Membrane Barriers to Blood-Tissue Transport of Potassium and Rubidium in Mammalian Skeletal Muscle

By R. M. Sheehan and E. M. Renkin

ABSTRACT

Blood-tissue transport of 42K, 86Rb, and 22Na was studied in perfused gracilis muscles of dogs to ascertain the influence of capillary wall, interstitial space, and muscle cell membranes on overall transport kinetics. Single-injection and continuous-infusion techniques were used, with T-1824 or 51Cr-hemoglobin as nondiffusible references. Results were as follows: (1) Extraction (E) of the diffusible solutes was incomplete even in the earliest samples of venous outflow. (2) E_Rb equaled E_K in the first few samples, but later, as both declined, the ratio of E_Rb to E_K became less than one; stable ratios between 0.6 and 0.8 were established in different experiments. (3) E_Na was initially slightly less than E_Rb or E_K but declined much more sharply in successive samples. (4) Steady-state E_Rb and E_K decreased with increasing blood flow but maintained a constant ratio. Vasomotion sometimes changed E_Rb and E_K but had little effect on their ratio. Local anesthetics (procaine or cocaine) brought the ratio of E_Rb to E_K closer to one. Analysis in terms of capillary and interstitial barriers, which do not discriminate between K, Rb, or Na, in series with a muscle cell membrane barrier, which does, leads to the following conclusions. (1) For K, the nondiscriminating barrier (mainly capillary wall) offers about 70% of the total resistance to transport and the discriminating barrier (cell membrane) about 30%. (2) For Rb, each barrier offers half the total resistance. (3) The resting muscle cell membrane is 2.5 times more permeable to K than to Rb. Local anesthetics reduce cell permeability to both ions and decrease this ratio. (4) For Na, resistance at the capillary wall is about 1.3 times that for K or Rb. Resistance at the cell membrane is, of course, very much greater.

KEY WORDS 42K, 86Rb, 22Na permeability muscular contraction single-injection, multiple-tracer method continuous-infusion method capillary extraction ratio permeability-surface area product procaine norepinephrine dog gracilis muscle

When blood containing a diffusible substance passes through the capillary bed of a tissue, a certain fraction (E) of the substance is removed and the remainder appears in the venous effluent (1–3). On the basis of a simple model of blood-tissue exchange, E can be shown to depend on capillary blood flow (Q) and on a "permeability-surface area product" (PS) of the diffusion barrier (4, 5). During continuous intra-arterial infusion of 42K or 86Rb in mammalian skeletal muscles, the...
concentration of tracer in venous blood rises rapidly to a plateau and only slowly thereafter. Values of $E$ obtained from venous plateau levels in skeletal muscle were used by Renkin (4) to calculate blood-tissue PS for $^{42}$K and later by Renkin and Rosell (6, 7) to calculate PS for $^{86}$Rb. The values for Rb were consistently smaller than those for K. Since there are good theoretical reasons to believe that there is little discrimination between these two ions either at the capillary wall or in the interstitial fluid (8), it appears that the difference between the PS's for $^{86}$Rb and $^{42}$K might well be due to the influence of the muscle cell membrane. Indeed, it was assumed by Conn and Robertson (9) that for cardiac muscle the cell membrane was the limiting factor in blood-tissue K exchange; Downey and Kirk (10) have shown that the K-specific activity of cardiac lymph is close to equilibrium with venous plasma, a fact which supports the cell membrane as the principal barrier. Yudilevich et al. (11), by incorporating a nonpermeating tracer in the infusion mixture, were able to measure $E$ for $^{86}$Rb in mammalian skeletal muscle for early-transit venous samples as well as for the plateau. They found that plateau extraction was half to two-thirds the maximum determined for early samples. In such early samples, $E$'s for $^{22}$Na and $^{86}$Rb differed only in proportion to their diffusion coefficients. Since muscle cell membranes are much less permeable to Na than to Rb and K, it appears that the "initial" diffusion barrier must reside principally in the capillary wall, and the plateau or "steady-state" barrier comprises both capillary wall and cell membrane. The fact that plateau $E_{nb}$ is a substantial fraction of initial $E_{nb}$ suggests that a large part of the steady-state barrier for Rb (and therefore also for K) is outside the cell membrane.

In the present work, we have examined the degree to which the capillary walls, interstitial space, and cell membranes contribute to the overall blood-tissue barrier in mammalian skeletal muscle. We have extended the earlier experiments of Renkin (4, 12) and Renkin and Rosell (6, 7) using $^{42}$K and $^{86}$Rb either simultaneously or alternately in the same preparation, with both the continuous-infusion and the single-injection methods. In a few experiments, we used $^{22}$Na as well, so as to have an indicator to which cell membrane permeability is low. In some experiments we altered capillary surface area by producing vasoconstriction with norepinephrine or vasodilatation by muscular contraction. In other experiments, we applied the local anesthetics procaine and cocaine to modify cell membrane permeability to K.

In many of our continuous-infusion experiments, we used a nonpermeating reference tracer to permit calculation of early-transient as well as steady-state extraction ratios (11). From experiments without such an indicator for washout dilution, only plateau extractions could be obtained. In our single-injection experiments, we were able to resolve only the early-transient data. The tail of the venous concentration curve is very long and flat in resting skeletal muscle, and recovery of injected tracers was incomplete within our period of collection. To support our partition of the resistance to blood-tissue diffusion based on analysis of continuous-infusion experiments with a nondiffusible reference, we have also used data obtained from partial analysis of continuous-infusion experiments without a reference and of single-injection experiments. It should be noted that the distinction we have thus made in the usage of single-injection and continuous-infusion experiments is not theoretically inherent in the two methods, since they contain, potentially, the same information (2). It is a consequence of purely practical limitations of their application to resting skeletal muscle, which normally has a low rate of blood flow per unit mass. For the dog heart, Ziegler and Goresky (13) have been able to analyze nearly complete output curves after single injections in which $^{86}$Rb and $^{22}$Na or $^{14}$C-sucrose were used as tracers and to establish that about half the total resistance to Rb was situated at the capillary wall and half at the cell membrane.
Methods

Mongrel dogs (9-15 kg) were anesthetized with sodium pentobarbital, 30 mg/kg, iv, supplemented as required. The left gracilis muscle was isolated as described elsewhere (4) and perfused at constant flow with an oil-displacement perfusion system (6, 14) or a Sigmanator pump drawing arterial blood from the dog. Perfusion pressure was measured with a strain-gauge manometer. The venous outflow from the muscle passed through a coil of polyethylene tubing in a scintillation counter with a 6-mm anthracene crystal for beta-particle counting and thence to a drop counter for monitoring of blood flow. Electrical outputs of the manometer, scintillation counter, and drop counter were recorded on a polygraph (Grass 3A). The mixed nerve supplying the muscle was cut and placed on electrodes for stimulation, if desired, by a Grass S-4 stimulator. To produce muscular contractions without activating vasomotor fibers, square pulses of 0.1-msec duration and just supramaximal for the motor fibers were used. Drugs (norepinephrine, chloral hydrate, cocaine, and procaine) were administered by continuous infusion into the arterial inflow line.

In the muscles perfused with the oil pump, the blood in the arterial reservoir was labeled with isotope at the beginning of the perfusion. In other experiments, isotopes were introduced into the arterial line upstream of the perfusion pump as either a continuous infusion or a rapid injection of 0.2-0.5 ml of mixed isotope solution. In addition to the diffusible isotopes, the solution contained a nondiffusible indicator, either T-1824 or 51Cr-labeled hemoglobin (15).

The experiments consisted of starting the infusion or making the injection and then following the time course of venous activity of the individual diffusible isotopes and of the nondiffusible reference. As soon as arrival of radioactivity on the venous side was signaled by the output of the scintillation counter, collection of venous samples was started. For the first few minutes of both the continuous-infusion and the single-injection experiments, all of the venous blood was collected, at first in samples of 5-10 drops each and then in samples of 10-20 drops each. After about 4 minutes, or after the scintillation counter indicated that changes of radioactivity with time were becoming less rapid, samples were taken every 20 or 30 seconds and then every minute. For the single injections, sample collection was stopped when the scintillation counter reading had fallen nearly to zero. In the continuous-infusion experiments, after the plateau of venous radioactivity had been reached as judged from the scintillation counter trace, various test procedures were started; further samples were taken when the scintillation counter trace had again become steady after the changes produced by the test. At the end of the experiment, samples for analysis of the arterial levels of isotopes were obtained by connecting the arterial cannula directly to the scintillation counter.

The arterial and venous samples were analyzed for isotopes and nondiffusible indicator as follows.

1. T-1824: after mixing the venous sample thoroughly, 1.2 ml was pipetted into a colorimeter tube; 3 ml of saline was added, the tubes were centrifuged, and the optical density of the supernatant fluid was read at 620 mu with a Coleman spectrophotometer.

2. For two experiments in which 86Rb and 42K were used alternately, the venous activity of the diffusible isotope was given directly by the scintillation counter.

3. When we had mixtures of two or more isotopes, we analyzed them by using their different gamma-ray energies and different half-lives. The details of the method and calculations are given by Yudilevich and Martin de Julian (16). The calculations were done with a digital computer. For the oil-pump experiments, the amounts of isotopes in the plasma were obtained by making corrections for uptake by red cells, as described by Renkin and Rosell (6). In the experiments in which the isotope mixture was added to the blood just before it entered the muscle (i.e., the Sigmanator-pump experiments), it was assumed that all the isotopes in a given blood sample were in the plasma.

The amounts of isotopes and T-1824 in the samples were expressed as a proportion of the isotope standards or T-1824 standard corrected for decay to the time that the perfusion started (i.e., normalized to amount injected as unity). Let these proportions be C'o„ for venous and arterial blood, respectively, where i = 86Rb, 42K, 22Na, or the reference, according to whether C'o and C'a are for 86Rb, 42K, 22Na, or the nondiffusible indicator. The venous levels of the various isotopes and of the nondiffusible tracer were then expressed as a proportion of their arterial activities or concentrations, C'o„ = C'o„/C'a„. The dilution of the venous blood by unlabeled blood is C'o„/C'o„ thus the activities (Vj) that the diffusible isotopes would have in the venous blood if there were no dilution of the labeled arterial blood are given by:

\[ V_j = C_o/j / (C_{o ref}/C_{a ref}) \]  

(1)

(See figures 1-3 for specific examples of this usage.) Absolutes venous activity levels for various tracers (C'o„) are plotted in Figure 3A. Venous activities normalized to arterial levels (C'o„) are plotted in Figures 1A and 2A. For the

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nondiffusible tracers (T-1824 or ⁵¹Cr-Hb) \( \frac{C_{V}}{C_{E}} \) approaches unity during the wash-in phase because of the initial dilution of injectate by unlabeled blood in the vessels. For diffusible tracers, the rising phase of \( C_{E} \) shows the combined influence of dilution and diffusion loss. Venous activities of diffusible tracers corrected for dilution (\( V_{J} \)) are plotted in B of the three figures.

In the papers of Yudilevich et al. (3, 11, 15, 16), \( V_{J} \) is called a "Cd/Cr" ratio, where \( d \) stands for diffusible and \( r \) for reference tracer, and \( Cd \) and \( Cr \) are normalized so that the amount injected equals unity. For the single-injection experiments, we calculated the apparent extraction ratio, \( E' \), for Rb, K, and Na, where \( E' = 1 - V_{J} \) since accumulation of isotopes in the tissue was minimal. For the continuous-infusion experiments, we also calculated the true extraction ratio, \( E \), for ⁸⁶Rb and ⁴²K. \( E \) is the amount of the given isotope removed compared with the total amount that could be removed:

\[
E = \frac{(1 - V)}{(1 - T)},
\]

where \( T \) is the tissue specific activity of the isotope as a proportion of its arterial level. ⁸⁶Rb and ⁴²K are diluted in the muscle cell ³⁹K pool, which is very large compared with the vascular pool (there is approximately 400-600 times as much extra- as intravascular ³⁹K in muscle). \( T_{K} \) and \( T_{Rb} \) were estimated at any given time either by the method of Renkin and Rosell (6) or by calculating the amount of either ⁴²K or ⁸⁶Rb removed from the blood up to that time and assuming that this amount was diluted in the total tissue ³⁹K pool; the latter method was used in all except the alternate ⁴²K and ⁸⁶Rb experiments. In either case, \( T \) was usually below 10%. Whatever method was used for calculating \( T \) made little difference to the relationships between \( E_{Rb} \) and \( E_{K} \) reported below. There is no large sink for ⁴⁴Na in muscle (extravascular Na is approximately five times intravascular) and so \( T_{Na} \) rises rapidly, and calculation of \( T_{Na} \) at any particular time is hazardous. We have presented our results for ⁴⁴Na in terms of the apparent extraction, \( E'_{Na} = (1 - V_{Na}) \).

Permeability-surface area products were calculated from transient and plateau data according to the formula (4, 5)

\[
PS = \frac{Q_{p} \ln(1 - E)}{1 - E},
\]

where \( Q_{p} \) is the plasma flow in ml/(min \times 100 g). We shall consider values calculated from plateau \( E \)'s measured in single-injection experiments or \( E \)'s measured before plateau of continuous-infusion experiments, according to the usage of Yudilevich et al. (11, 15). Their significance and the relation of initial PS values to the permeability-surface area product for the capillary wall (\( PS_{cap} \)) will be discussed subsequently. Vascular resistance was calculated as the ratio of arterial pressure (\( pA \)) to total blood flow (assuming that venous pressure = 0) and expressed in mm Hg/ml-1 min \times 100 g.

### Results

**TRANSIENT PHASE (WASH-IN OF TRACERS)**

Figure 1 shows the results of an experiment in which ⁸⁶Rb and ⁴²K were infused simultaneously into the arterial blood; five successful experiments of this kind were made in as many preparations. Figure 1A shows the raw

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Cv data for the first 22 minutes of continuous infusion. T-1824 was used as the nondiffusible reference. During the first few minutes, its concentration in venous blood rose to that in arterial blood. The concentrations of $^{86}$Rb and $^{42}$K also rose but to levels below arterial. During the earliest part of wash-in, venous $^{86}$Rb and $^{42}$K were approximately equal, but as they approached their plateau, venous $^{86}$Rb climbed above $^{42}$K. This is reflected in Figure 1B which shows $V_{\text{in}}$ and $V_{K}$, the venous concentrations corrected for dilution with unlabeled blood according to Eq. 1, and the calculated ratio of $E_{\text{in}}$ to $E_{K}$. For the first three samples, $V_{\text{rb}}$ and $V_{K}$ rose together from 0.28 to 0.48 while the ratio of $E_{\text{in}}$ to $E_{K}$ remained at unity. Then, as $V_{\text{rb}}$ and $V_{K}$ rose to plateau levels of 0.68 and 0.58 respectively, the ratio of $E_{\text{in}}$ to $E_{K}$ fell to a stable value of 0.76.

Figure 2 shows the results of an infusion experiment in which $^{42}$K, $^{86}$Rb, and $^{22}$Na were used simultaneously as diffusible tracers and $^{51}$Cr-hemoglobin as the nondiffusible reference. The time scale of sampling was more rapid than it was in Figure 1, and the graphs show mainly the rising phase of the venous concentration curve. Due to the small samples and greater number of isotopes to be discriminated, the statistics of individual data points are not so satisfactory. The data for $^{86}$Rb and $^{42}$K are similar to those in Figure 1. The curve for $V_{Na}$ started off a little higher than did those for $V_{K}$ or $V_{in}$, (the K concentration of the third sample was entirely out of line with the other data and is best ignored) but ran parallel to them until just after 2 minutes when the ratio of $E_{\text{rb}}$ to $E_{K}$ started falling below unity. $V_{\text{rb}}$ continued to rise beyond this point, but $V_{\text{in}}$ and $V_{K}$ remained low. The plots show that the ratio of $E_{Na}$ to $E_{K}$ stayed above 0.8 for the first 2 minutes of infusion, but then fell rapidly, approaching zero as the more limited distribution volume of Na equilibrated with arterial plasma. In this experiment, in contrast to the one illustrated in Figure 1, $E_{K}$ and $E_{\text{rb}}$ did not fall significantly during the period in which they were equal. The two experiments chosen for illustration represent the extremes encountered within our limited experience. In most experiments $E_{K}$ and $E_{\text{rb}}$ decreased to about 0.7 their initial values in the few samples collected before their ratio fell below 1.

The results of a single injection are shown in Figure 3: the injection mixture contained $^{86}$Rb, $^{42}$K, and T-1824. Figure 3A shows venous outflow concentrations on a logarithmic scale. These were nearly equal for $^{86}$Rb and $^{42}$K during the rising phase of the curves, but showed gradual and progressive separation during the falling phase, with $^{86}$Rb outflow always greater than that of $^{42}$K. After 2.7 minutes, venous concentration of $^{86}$Rb exceeded that of T-1824, but $^{42}$K remained consistently lower. The corresponding values of $V_{\text{rb}}$ and $V_{K}$ and the ratio of $E_{\text{rb}}$ to $E_{K}$ are shown in Figure 3B. As in the continuous-infusion experiment, the ratio of $E_{\text{rb}}$ to $E_{K}$...
remained close to unity during the early part of the curve (the entire rising phase in this case) and then fell. However, the fall was much greater during the washout of a single injection than during a sustained infusion (Fig. 1). In this experiment, as in the one shown in Figure 2, $E_K$ and $E_{Rb}$ did not decline while their ratio remained constant. In the other successful single-injection experiment, which incorporated $^{22}\text{Na}$ in the injection mixture as well as $^{86}\text{Rb}$ and $^{42}\text{K}$, $E_{Rb}$ and $E_K$ fell from 0.75 to 0.52 in this period and $E_{Na}$ from 0.55 to 0.41, maintaining a ratio of about 0.8, until it started to fall more sharply.

Under the heading Transient Data in Table 1 are summarized certain data obtained in these and similar experiments. Mean $E$'s and $PS$ values calculated from them by Eq. 3 are given for the initial period in which these two parameters are identical (within $\pm 2\%$) for Rb and K. During this period, $E$ values decreased; the extent of the decrease is indicated by the numbers in parentheses. Lumped $PS$ values, calculated from plateau $E$ measured later in the course of the same infusion experiments are listed under the heading Plateau Data.

**PLATEAU PHASE (QUASI STEADY STATE)**

In ten perfused muscles, after the plateau of a continuous infusion of $^{86}\text{Rb}$ and $^{42}\text{K}$ had been reached (Figs. 1 and 2), variation of perfusion rate and various other procedures were carried out while the infusion continued to test their influence on the interrelation between steady-state $E$'s for the two isotopes and on the $PS$ values calculated from them. These are designed $PS_i$ to distinguish them from $PS$ values calculated from transient measurements. In two additional experiments, $^{86}\text{Rb}$ and $^{42}\text{K}$ were used alternately. Figure 4 illustrates one experiment of four in which the effect of changing blood flow was studied. The data for four experiments in which blood flow was varied are given in Table 2. When $Q$ was increased, plateau $E$'s for both K and Rb fell, without exception, as described previously by Renkin (4) and by Friedman (17). There was a tendency of $PS_i$ for both Rb and K to increase with increasing flow. In each experiment, however, the ratio of $PS_{Rb}$ to $PS_{K}$ stayed nearly constant with no consistent relation to flow. The mean value of this ratio varied from 0.68 to 0.79 in these experiments (the range of control values in all experiments was 0.56 to 0.79, see Tables 3 and 4).

In eight simultaneous experiments with Rb and K, we infused procaine (in one also cocaine) during the plateau to see if an agent
### TABLE 1

**Transient Extraction Ratios** ($E'$) **Observed while $E'_{K,b}$ = $E'_{Na}$ and PS Values Calculated from Them**

<table>
<thead>
<tr>
<th>Expt</th>
<th>$Q_o$ (ml/min x 100 g)</th>
<th>$E'_{K,b}$</th>
<th>$PS_{Na/PS_{b,b}}$ (ml/min x 100 g)</th>
<th>$E'_{Na}$</th>
<th>$PS_{Na}$ (ml/min x 100 g)</th>
<th>$PS_{Na}/PS_{b,b}$</th>
<th>Plateau data</th>
<th>$PS_{b,b}$ (ml/min x 100 g)</th>
<th>$PS_{b,b}/PS_{Na}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>7.4</td>
<td>0.61</td>
<td>(0.72 → 0.52)</td>
<td>7.2</td>
<td>(9.4 → 5.4)</td>
<td>2.9</td>
<td>4.0</td>
<td>0.72</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>7.0</td>
<td>0.44</td>
<td>(0.55 → 0.38)</td>
<td>4.2</td>
<td>(3.6 → 3.4)</td>
<td>2.0</td>
<td>2.7</td>
<td>0.74</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>5.3</td>
<td>0.70</td>
<td>(0.75 → 0.66)</td>
<td>6.5</td>
<td>(7.4 → 5.7)</td>
<td>2.8</td>
<td>4.2</td>
<td>0.67</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>3.0</td>
<td>0.57</td>
<td>(0.68 → 0.47)</td>
<td>2.6</td>
<td>(3.4 → 1.9)</td>
<td>0.53</td>
<td>2.4</td>
<td>0.92</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>3.2</td>
<td>0.71</td>
<td>(0.73 → 0.70)</td>
<td>4.0</td>
<td>(4.2 → 3.8)</td>
<td>0.59</td>
<td>2.8</td>
<td>0.70</td>
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<tr>
<td><strong>MEAN</strong></td>
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<td></td>
<td></td>
<td></td>
<td>4.9</td>
<td>2.6</td>
<td>0.81</td>
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**Continuous-Infusion Experiments**

<table>
<thead>
<tr>
<th>Expt</th>
<th>$Q_o$ (ml/min x 100 g)</th>
<th>$E'_{K,b}$</th>
<th>$PS_{Na/PS_{b,b}}$ (ml/min x 100 g)</th>
<th>$E'_{Na}$</th>
<th>$PS_{Na}$ (ml/min x 100 g)</th>
<th>$PS_{Na}/PS_{b,b}$</th>
<th>Plateau data</th>
<th>$PS_{b,b}$ (ml/min x 100 g)</th>
<th>$PS_{b,b}/PS_{Na}$</th>
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</thead>
<tbody>
<tr>
<td>7</td>
<td>3.5</td>
<td>0.64</td>
<td>(0.61 → 0.65)</td>
<td>3.6</td>
<td>(3.3 → 3.7)</td>
<td>2.3</td>
<td>0.68</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>3.2</td>
<td>0.62</td>
<td>(0.75 → 0.52)</td>
<td>3.4</td>
<td>(4.5 → 2.3)</td>
<td>0.50</td>
<td>2.3</td>
<td>0.68</td>
<td></td>
</tr>
<tr>
<td><strong>MEAN (all expts)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.5</td>
<td>2.5</td>
<td>0.77</td>
<td></td>
</tr>
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</table>

**Single-Injection Experiments**

The entries are arithmetic means of all measurements meeting the criterion, and the range is given in parentheses. In the last three columns, plateau PS values for K and Rb and their ratios are listed for the same continuous infusions. See text for abbreviations.
known to alter muscle cell membrane permeability to K (18) would influence $PS_i$ values for K and Rb and their ratio. One of these experiments is illustrated in Figure 5. Infusion of procaine at 0.14 mg/(min×100 g) while arterial levels of $^{86}$Rb and $^{42}$K were constant resulted in an elevation of venous levels of both tracers. But $V_K$ was elevated more than $V_{Rb}$ and thus the ratio of their $E$'s and $PS$'s was brought closer to one. The change might progress over several minutes and was partially reversible after cessation of procaine infusion. The results of all experiments are given in Table 3. In four of them, chloral hydrate was infused before and during the procaine infusion to produce maximum vasodilation in an effort to minimize the vasoconstriction often produced by procaine alone. Phenoxybenzamine, tried in one experiment, did not prevent this. In all experiments $PS_{infb}$ and $PS_{infb}$ both decreased after procaine, irrespective of whether vascular resistance increased. In all but one experiment, the ratio of $PS_{infb}$ to $PS_{infb}$ increased but in no case did it exceed 0.87.

Table 4 shows three experiments on the effect of intra-arterial norepinephrine infusion on $PS_{infb}$ and $PS_{infb}$ at constant blood flow. As has been shown previously, norepinephrine produced an increase in vascular resistance and a decrease in $PS_{infb}$ and $PS_{infb}$ (19). The ratio of Rb to K was increased slightly (10–20%) in three experiments and decreased in the fourth. Also listed in Table 4 are the results of three experiments on the effects of metabolic vasodilation (muscular contraction) on $PS_{infb}$ and $PS_{infb}$. The procedure used was like that described by Renkin et al. (20) except that $^{86}$Rb and $^{42}$K were present simultaneously in the arterial blood. After the
plateau of venous radioactivity had been reached, the gracilis nerve was stimulated with 0.1-msec pulses of 2-4 v, at frequencies of 0.5-2/sec. At constant blood flow, £Kb and £K both increased. Calculated increases in PS,w, and PSIK were similar to those reported earlier and nearly proportional: there were no substantial or consistent changes in the ratio of PS,w to PSIK.

**Discussion**

**EARLY EXTRACTION OF 32Rb, 42K, AND 36Na**

We will first consider why the isotopes are not completely extracted from the blood. This might be because the isotope in blood is brought to equilibrium with that in the tissue. At the flow rates used in these experiments, at least half the total 39K of muscle is readily exchangeable with blood (12). By calculating the amount of 42K or 86Rb removed from blood up to the time of an observation from the plasma flow and arteriovenous difference, and dividing this by the exchangeable K pool in the muscle, one can estimate the specific activity of 42K or 86Rb in the tissue. In experiments of 1 or 2 hours in duration, this could have reached barely 10% of the arterial specific activity and at shorter times, proportionately less. It follows that these isotopes do not come into equilibrium with all exchangeable tissue K.
TABLE 3

Influence of Local Anesthetics on Plateau Extraction of $^{42}$K and $^{86}$Rb

<table>
<thead>
<tr>
<th>Expt</th>
<th>Procedure</th>
<th>$Q_b$ (ml/min $\times 100$ g)</th>
<th>$gA/Q_b$ (mm Hg min $100$ mg)</th>
<th>$E_x$</th>
<th>$E_{in}/E_x$</th>
<th>$P_{SlK}$ (ml/min $\times 100$ g)</th>
<th>$P_{Sln}/P_{SlK}$</th>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Control*</td>
<td>8.6</td>
<td>2.9</td>
<td>0.46</td>
<td>0.67</td>
<td>5.32</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td>Procaine (1) (2.6 mg/ml)</td>
<td>8.6</td>
<td>4.2</td>
<td>0.29</td>
<td>0.69</td>
<td>2.92</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td>Procaine (2) (2.6 mg/ml)</td>
<td>8.6</td>
<td>6.3</td>
<td>0.13</td>
<td>0.85</td>
<td>1.20</td>
<td>0.86</td>
</tr>
<tr>
<td>4</td>
<td>Control*</td>
<td>7.3</td>
<td>2.1</td>
<td>0.52</td>
<td>0.73</td>
<td>5.30</td>
<td>0.66</td>
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<tr>
<td></td>
<td>Procaine (1) (1.0 mg/ml)</td>
<td>7.3</td>
<td>2.4</td>
<td>0.42</td>
<td>0.81</td>
<td>3.94</td>
<td>0.78</td>
</tr>
<tr>
<td></td>
<td>Procaine (2) (1.0 mg/ml)</td>
<td>7.3</td>
<td>2.5</td>
<td>0.34</td>
<td>0.88</td>
<td>3.07</td>
<td>0.86</td>
</tr>
<tr>
<td>5</td>
<td>Control</td>
<td>8.4</td>
<td>12.6</td>
<td>0.32</td>
<td>0.78</td>
<td>3.28</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td>Procaine (1.2 mg/ml)</td>
<td>8.4</td>
<td>9.6</td>
<td>0.27</td>
<td>0.70</td>
<td>2.60</td>
<td>0.68</td>
</tr>
<tr>
<td>6</td>
<td>Control</td>
<td>7.3</td>
<td>7.8</td>
<td>0.30</td>
<td>0.77</td>
<td>2.63</td>
<td>0.72</td>
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<tr>
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<td>Procaine (1.7 mg/ml)</td>
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<td>1.97</td>
<td>0.82</td>
</tr>
<tr>
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<td>Control</td>
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<td>9.7</td>
<td>0.44</td>
<td>0.84</td>
<td>3.07</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>Procaine (1.0 mg/ml)</td>
<td>5.3</td>
<td>22.7</td>
<td>0.32</td>
<td>0.91</td>
<td>2.07</td>
<td>0.87</td>
</tr>
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<td>11.2</td>
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<td>4.5</td>
<td>18.0</td>
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<td>0.74</td>
<td>3.50</td>
<td>0.65</td>
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<td>2.9</td>
<td>0.41</td>
<td>0.66</td>
<td>3.40</td>
<td>0.59</td>
</tr>
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<td>3.7</td>
<td>0.29</td>
<td>0.69</td>
<td>2.18</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td>Recovery (partial)</td>
<td>6.4</td>
<td>2.9</td>
<td>0.32</td>
<td>0.69</td>
<td>2.50</td>
<td>0.64</td>
</tr>
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<td></td>
<td>Cocaine (0.8 mg/ml)</td>
<td>6.4</td>
<td>1.7</td>
<td>0.18</td>
<td>0.78</td>
<td>1.28</td>
<td>0.75</td>
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<tr>
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<td>Control*</td>
<td>3.2</td>
<td>3.6</td>
<td>0.63</td>
<td>0.71</td>
<td>3.17</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td>Procaine (1.4 mg/ml)</td>
<td>3.2</td>
<td>3.5</td>
<td>0.46</td>
<td>0.74</td>
<td>1.98</td>
<td>0.68</td>
</tr>
</tbody>
</table>

Procaine or cocaine was infused intra-arterially at the plasma concentration indicated in parentheses.
* Treatment with chloral hydrate, 2-3 mg/ml plasma.
† Treatment with phenoxybenzamine, 0.2 mg/100 g muscle, ia.

In the case of $^{22}$Na, however, the exchangeable pool of tissue $^{22}$Na is much smaller and must proceed a considerable part of the way towards equilibrium in a few minutes. This is the basis of our explanation and that of Yudilevich et al. (11) for the rapid fall of $E_{Na}$ in successive venous samples, compared with Rb or K. However, this argument cannot apply to the earliest samples of a series, which should represent exchange with as yet uncontaminated interstitial Na.

There are two basic mechanisms which could produce a venous activity or concentration of diffusible tracer greater than that in the tissue. (1) There may be shunt channels through the capillary bed in which there is no exchange in parallel with exchange channels in which equilibration is complete (17). (2) Exchange may be incomplete in the individual channels (4). Of course, a combination of these two mechanisms is possible, and we are not able to distinguish categorically between them. However, we think the evidence is against the pure shunt mechanism. (For a discussion of earlier evidence, see Renkin et al. (20), Friedman (17), and Chien (21).) It is difficult to imagine how such a mechanism could account for different venous levels for $^{86}$Rb and $^{42}$K, since they presumably enter the same pool of $^{38}$K in the exchange channels, or for different initial levels of Na relative to K and Rb, which are then equal. Similar arguments have been put forward for the different $E$'s of antipyrine and urea, both of which equilibrate with total tissue water (22). Friedman’s experimental results on the effects of blood flow on $E_{in}$, which he has interpreted in terms of partition of flow between “nutritional” capillaries and shunts, are actually closely similar to ours. The fact that a plot of log $E$ vs. $Q$ extrapolates towards complete extraction at zero flow is a direct prediction of the incomplete equilibration hypothesis. What is more difficult in Friedman’s results to explain by this mechanism is that $E$ is little affected by flow during the
**TABLE 4**

<table>
<thead>
<tr>
<th>Expt</th>
<th>Procedure</th>
<th>$Q_p$</th>
<th>$pA/Q_p$</th>
<th>$E_K$</th>
<th>$E_{Na}/E_K$</th>
<th>$P_{Sb}$</th>
<th>$P_{Sb}/P_{Sb}/Sb$</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>Control</td>
<td>7.3</td>
<td>7.9</td>
<td>0.33</td>
<td>0.79</td>
<td>2.92</td>
<td>0.75</td>
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<tr>
<td></td>
<td>NE (4.2 µg/[min × 100 g])</td>
<td>7.3</td>
<td>19.7</td>
<td>0.23</td>
<td>0.82</td>
<td>1.91</td>
<td>0.81</td>
</tr>
<tr>
<td></td>
<td>Recovery</td>
<td>7.3</td>
<td>10.0</td>
<td>0.28</td>
<td>0.86</td>
<td>2.42</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>7.3</td>
<td>7.9</td>
<td>0.37</td>
<td>0.79</td>
<td>3.37</td>
<td>0.74</td>
</tr>
<tr>
<td>7</td>
<td>Control</td>
<td>5.3</td>
<td>13.6</td>
<td>0.34</td>
<td>0.82</td>
<td>2.22</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>NE (1.8 µg/[min × 100 g])</td>
<td>5.3</td>
<td>23.6</td>
<td>0.26</td>
<td>0.77</td>
<td>1.59</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>5.3</td>
<td>12.5</td>
<td>0.36</td>
<td>0.80</td>
<td>2.38</td>
<td>0.86</td>
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<tr>
<td>12</td>
<td>Control</td>
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<td>3.0</td>
<td>0.39</td>
<td>0.67</td>
<td>4.26</td>
<td>0.61</td>
</tr>
<tr>
<td></td>
<td>NE (3.5 µg/[min × 100 g])</td>
<td>8.7</td>
<td>13.8</td>
<td>0.26</td>
<td>0.73</td>
<td>2.61</td>
<td>0.70</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>8.7</td>
<td>4.1</td>
<td>0.35</td>
<td>0.71</td>
<td>3.74</td>
<td>0.67</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>5.3</td>
<td>2.0</td>
<td>0.51</td>
<td>0.74</td>
<td>3.76</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>NE (1.8 µg/[min × 100 g])</td>
<td>5.3</td>
<td>18.5</td>
<td>0.31</td>
<td>0.84</td>
<td>1.96</td>
<td>0.81</td>
</tr>
<tr>
<td>6</td>
<td>Control</td>
<td>7.3</td>
<td>7.9</td>
<td>0.37</td>
<td>0.79</td>
<td>3.37</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td>MC (1/sec)</td>
<td>7.3</td>
<td>6.2</td>
<td>0.50</td>
<td>0.77</td>
<td>3.58</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>7.3</td>
<td>7.8</td>
<td>0.30</td>
<td>0.73</td>
<td>2.63</td>
<td>0.70</td>
</tr>
<tr>
<td>8</td>
<td>Control</td>
<td>7.4</td>
<td>9.3</td>
<td>0.47</td>
<td>0.74</td>
<td>4.66</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>MC (0.5/sec)</td>
<td>7.4</td>
<td>5.8</td>
<td>0.54</td>
<td>0.72</td>
<td>6.07</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td>MC (1.0/sec)</td>
<td>7.4</td>
<td>3.8</td>
<td>0.52</td>
<td>0.81</td>
<td>5.40</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td>Recovery</td>
<td>7.4</td>
<td>7.8</td>
<td>0.43</td>
<td>1.00</td>
<td>4.14</td>
<td>1.00</td>
</tr>
<tr>
<td>12</td>
<td>Control</td>
<td>8.7</td>
<td>3.9</td>
<td>0.38</td>
<td>0.69</td>
<td>4.18</td>
<td>0.62</td>
</tr>
<tr>
<td></td>
<td>MC (1/sec)</td>
<td>8.7</td>
<td>2.2</td>
<td>0.57</td>
<td>0.72</td>
<td>7.30</td>
<td>0.63</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>8.7</td>
<td>3.8</td>
<td>0.40</td>
<td>0.70</td>
<td>4.43</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td>MC (2/sec)</td>
<td>8.7</td>
<td>1.6</td>
<td>0.57</td>
<td>0.72</td>
<td>7.30</td>
<td>0.63</td>
</tr>
<tr>
<td></td>
<td>Recovery</td>
<td>8.7</td>
<td>3.0</td>
<td>0.39</td>
<td>0.67</td>
<td>4.26</td>
<td>0.61</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>5.3</td>
<td>4.0</td>
<td>0.47</td>
<td>0.79</td>
<td>3.34</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>MC (1/sec)</td>
<td>5.3</td>
<td>1.4</td>
<td>0.61</td>
<td>0.79</td>
<td>4.98</td>
<td>0.69</td>
</tr>
<tr>
<td></td>
<td>Recovery</td>
<td>5.3</td>
<td>2.0</td>
<td>0.51</td>
<td>0.74</td>
<td>3.76</td>
<td>0.68</td>
</tr>
</tbody>
</table>

NE was administered by intra-arterial infusion of amount indicated, MC by stimulation of gracilis nerve at frequency indicated.

vasodilatation after release of arterial occlusion. However, he plotted the maximum $E$ calculated from the transient minimum venous levels occurring after a period of zero flow. One might reasonably expect such a venous level to be dependent on the diffusion characteristics of the vascular bed during the static period rather than on subsequent restoration of flow. The situation is very much like a stopped-flow experiment on the kidney. We conclude that there must be at least some (i.e., a substantial proportion of) channels present with incomplete equilibration of $^{86}$Rb, $^{42}$K, and $^{22}$Na in the flow ranges studied. It is not necessary that there be shunt channels to explain our results, but we cannot rule them out on the evidence presented here.

**PLATEAU EXTRACTIONS OF RB AND K**

What could be responsible for the difference between plateau extractions of Rb and K? In an incompletely equilibrating capillary a difference in venous levels of $^{86}$Rb and $^{42}$K implies that the resistance to diffusion of the total path from plasma to cell interior must be different for the two ions. This path includes capillary endothelium, interstitial space, and muscle cell membrane. There is much evidence that lipid-insoluble substances pass across the capillary wall between the cells rather than by going through them (8). Our observation that the initial extraction of $^{22}$Na is only slightly less than that for $^{86}$Rb and $^{42}$K agrees with this idea: if these ions passed through the endothelial cells and if the ionic permeabilities of their cell membranes are like $^{86}$Rb, $^{42}$K, and $^{22}$Na in the flow ranges studied.
other cells, one would expect initial $E_{Na}$ to be much less than $E_K$ or $E_{Rb}$ (11). In passive diffusion between the endothelial cells, it is most unlikely that there would be any discrimination between Rb and K since their free diffusion coefficients in water are practically identical, and indeed, over the earliest phase of the wash-in, their extractions are the same.

The next site at which there might be discrimination between Rb and K is the interstitial fluid. It is becoming increasingly apparent that its properties are not simply those of a watery solution of protein but more closely resemble a gel with interspaces of molecular size which can restrict distribution of materials (23). Ogston and Sherman (24) have shown that dilute solutions of hyaluronic acid can decrease rates of diffusion by factors up to several fold for large molecules, less for small. Since the process of restriction is largely a mechanical one, it seems unlikely that it would discriminate between two so similar ions. It seems more in accordance with known facts to seek to explain the difference in blood-tissue $E$'s for $^{86}$Rb and $^{42}$K by a difference in their transport rates across the muscle cell membranes. Such a difference has been found for frog sartorius (25-27), but to our knowledge similar measurements have not been made in mammalian skeletal muscle. If a difference in muscle cell permeability to Rb and K is to have an effect on their extraction from the bloodstream, it follows that the resistance of the muscle cell membrane must form an appreciable part of the total resistance of the diffusion pathway. The effect of the cell membrane is seen only in the plateau or steady state of the infusion experiments: it is absent from the earliest phases, in which $E_{Rb}$ and $E_K$ are equal.

**SERIES BARRIERS TO TRANSPORT**

Without going into details of the geometry, we may represent capillary (cap), interstitial (isf), and cell membrane barriers as resistances in series,

$$R_{tot} = R_{cap} + R_{isf} + R_{cell}, \quad (4a)$$

where each $R$ is the reciprocal of the corresponding $PS$,

$$\frac{1}{PS_i} = \frac{1}{PS_{cap}} + \frac{1}{PS_{isf}} + \frac{1}{PS_{cell}}. \quad (4b)$$

$R_{tot}$ for $^{86}$Rb or $^{42}$K is obtained experimentally from their plateau $E$'s according to Eq. 3. Martin de Julian and Yudilevich (3) suggested that $R_{cap}$ may be evaluated from initial extraction ratios by the same equation. Our observations support this notion: (1) For the first few values obtained, $E_{Rb} = E_K$, and $E_{Na}$ is only slightly less. Such lack of restriction would be expected from capillary wall or interstitial fluid, or both, but not from cell membrane. (2) Though absolute values of $E$ may fall in samples after the first, as long as the ratio of $E_{Rb}$ to $E_K$ remains unity, the ratio of $E_{Na}$ to them both remains constant (Table 1). If the decrease in $E$ in this period were due to establishment of a gradient across the interstitial space, we should expect $E_{Na}$ to fall much faster than $E_K$ or $E_{Rb}$, since it cannot escape from the interstitium. Therefore, the barrier represented by these early samples must represent only the capillary wall. Back-diffusion of Na$^+$ from the interstitial space becomes important only after the ratio of $E_{Rb}$ to $E_K$ becomes less than unity, and then $E_{Na}$ falls more rapidly than $E_{Rb}$ or $E_K$ (Fig. 2).

We attribute the decrease in $E$'s observed in most experiments while $E_{Rb} = E_K$ to heterogeneity of capillary pathways in the perfused muscle. The earliest samples have higher $E$'s, indicative of higher ratios of $PS$ to $Q$, than those collected later. The degree of inhomogeneity varies greatly from experiment to experiment; those shown in Figures 1 and 2 represent extremes. On this assumption, we have taken averages of early $E$ values with $E_{Rb} = E_K$ for calculation of $PS_{cap}$. This practice differs from that of Yudilevich and his associates (3, 11, 15) which concentrates on the earliest samples. Our figures represent, therefore, a more sizable fraction of the capillary bed than theirs but still not all of it, because venous levels of reference tracer had not reached steady levels (in continuous-infusion experiments) before the ratio of $E_{Rb}$ to $E_K$ fell below unity. A more complete
representation of capillary pathways would probably lower the average $E$.

The interstitial barrier is lost somewhere between the early ($E_{RB} = E_K$) and plateau phases of the measurements. We can show, on the basis of a geometric model of the capillary and its surrounding (see Appendix) that unless diffusion in the interstitial fluid matrix is less than 1/100th of that in free solution or unless capillary-cell membrane diffusion distances exceed 20$\mu$, $R_{int}$ is negligible in comparison with $R_{cap}$ or $R_{cell}$. We may therefore reduce Eqs. 4a and 4b to their three significant terms:

$$R_{tot} = R_{cap} + R_{cell}, \quad (5a)$$

or

$$\frac{1}{P_S} = \frac{1}{P_{S_{cap}}} + \frac{1}{P_{S_{cell}}}. \quad (5b)$$

These equations are analogous to those used by Forster (28) in partition of total resistance to gas diffusion in the lung. $P_S$ is equivalent to the experimentally measured pulmonary diffusion coefficient and $P_{S_{cap}}$ to its membrane component.

In Table 5 are listed values for $R_{tot}$, $R_{cap}$, and $R_{cell}$ calculated by Eqs. 5a and 5b for the five continuous-infusion experiments of Table 1. Tabulated also are the ratios of cell membrane to total resistance and cell membrane exchange rates ($\rho$) for Rb and K calculated from $P_{S_{cell}}$ according to the relation (12),

$$P_{S_{cell}} = \frac{\rho}{[K]}, \quad (6)$$

where [K] is the concentration of carrier ion, $^{39}$K, for both isotopes, in plasma and interstitial fluid. (The fluxes measured for both tracers in these experiments are inflows. However, since muscle cell potassium remains practically constant, $^{39}$K outflow must equal $^{42}$K or $^{86}$Rb influx.) For K, the capillary wall presents 69% of the total resistance to blood-tissue transport, the muscle cell membranes 31%. For Rb, the resistance is more evenly divided, 47% capillary and 53% cell membrane. The cell membrane exchange rate of Rb is substantially less than for K. The ratio of $\rho_{Rb}$
to $\rho_K$, designated $\alpha$, is on the average 0.4, somewhat less than the ratio of 0.55 reported for frog sartorius (25-27).

**ANALYSIS OF STEADY-STATE RESULTS**

Assuming a fixed ratio ($\alpha$) of muscle cell membrane exchange rates for $Rb$ and $K$, we can calculate capillary and cell membrane exchange rates for these ions from simultaneous measurements of their steady-state transport. From Eq. 5a we write for simultaneous measurements,

$$\frac{1}{PS_{cap} K} = \frac{1}{PS_{cell} K} - \frac{1}{PS_{Rb} K}$$  \hspace{1cm} (7a)

$$\frac{1}{PS_{cell} Rb} = \frac{1}{PS_{cap} Rb} - \frac{1}{PS_{Rb} Rb}.$$  \hspace{1cm} (7b)

Since $PS_{cap} Rb = PS_{cap} K$,

$$\frac{1}{PS_{cell} Rb} = \frac{1}{PS_{cell} K} - \frac{1}{PS_{Rb} Rb}.$$  \hspace{1cm} (8)

Rearranging,

$$\frac{1}{PS_{cell} Rb} = \frac{1}{PS_{cell} K} + \left( \frac{1}{PS_{Rb} Rb} - \frac{1}{PS_{Rb} K} \right).$$  \hspace{1cm} (9)

The term in parenthesis is directly obtainable from experimental data. We shall represent it by the symbol $\Delta R_{Rb,K}$. Furthermore, the ratio of $PS_{cell} Rb$ to $PS_{cell} K = \alpha$ (see Eq. 6), and thus

$$PS_{cell} K = \left( \frac{1}{\alpha} - 1 \right) + \Delta R_{Rb,K}.$$  \hspace{1cm} (10a)

Expressed in resistance notation ($R = 1/PS$),

$$R_{cell} K = \Delta R_{Rb,K} + \left( \frac{1}{\alpha} - 1 \right).$$  \hspace{1cm} (10b)

This form is more convenient for calculations and is used in the tables. Corresponding values for $Rb$ are obtained with $\alpha$, and exchange rates ($\rho$) for the two ions by Eq. 6. Table 6 lists values of steady-state $R_{tot}$, $R_{cell}$, and $R_{cap}$ for $K$ and $Rb$ calculated for the experiments of Tables 1 and 2. For $\alpha$, we used the value 0.40, which is the average obtained from transient results in the first five experiments (Table 5). Because an average value of $\alpha$ was used, the partition of resistances for individual experiments differs slightly from that given in Table 5. The results

**TABLE 6**

Calculation of Capillary and Cell Membrane Resistances to $Rb$ and $K$ and Cell Membrane Exchange Rate for $K$ from Data Obtained in the Plateau Phase of Continuous Infusion Experiments

<table>
<thead>
<tr>
<th>Experiment</th>
<th>$R_{cell} K$</th>
<th>$R_{cell} Rb$</th>
<th>$R_{cap} Rb$</th>
<th>$R_{cap} K$</th>
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</thead>
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<tr>
<td>1</td>
<td>0.25</td>
<td>0.30</td>
<td>0.32</td>
<td>0.30</td>
</tr>
<tr>
<td>2</td>
<td>0.20</td>
<td>0.25</td>
<td>0.28</td>
<td>0.28</td>
</tr>
<tr>
<td>3</td>
<td>0.25</td>
<td>0.30</td>
<td>0.32</td>
<td>0.30</td>
</tr>
<tr>
<td>4</td>
<td>0.20</td>
<td>0.25</td>
<td>0.28</td>
<td>0.28</td>
</tr>
<tr>
<td>5</td>
<td>0.25</td>
<td>0.30</td>
<td>0.32</td>
<td>0.30</td>
</tr>
</tbody>
</table>

The ratio of cell membrane exchange rates for $Rb$ and $K$ ($\alpha$) is taken as 0.40 from our transient phase data (Table 5) or as 0.55, the value reported for frog skeletal muscle (25-27). For experiments 1, 2, 7, and 10, the mean of values obtained over a range of perfusion rates is given.
for these and for the other experiments in which no transient data were obtained are closely similar. For K, cell membrane resistance averages 0.27 (range 0.18 to 0.39) of total resistance, independent of plasma flow. For Rb, cell membrane resistance is 0.48 (range 0.35 to 0.62) of total resistance. Cell membrane exchange rate for K, calculated by Eq. 6, using a standard interstitial fluid concentration of 4 mEq/liter, averages 52 μEq/(min × 100 g muscle). There is some difference in this quantity from one experiment to another and a tendency toward low values at low plasma flows. The average of values measured at $Q_p$'s above 3.0 ml/(min × 100 g) is 58 μEq/(min × 100 g). Our estimate of $p_K$ for the dog gracilis muscle lies between the figure of 45 μEq/(min × 100 g) given by Creese (29) and that of 90 μEq/(min × 100 g) given by Calkins et al. (30), both for the rat diaphragm. If $\alpha$ is taken as 0.55 as for frog sartorius (25-27) instead of 0.4, the ratio of $R_{cell}$ to $R_{tot}$ is higher for both Rb and K (0.50 and 0.64, respectively) but $p_K$ falls to only 28 μEq/(min × 100 g) on the average, substantially less than the smaller of the two direct estimates for skeletal muscle in vitro. This may explain part of the difference (usually only qualitative) between such estimates of effective capillary surface and those based on measurement of capillary filtration coefficients, which presumably depend only on the capillary barrier (33, 34). A change in $P_{Scap}$ unaccompanied by any change of $P_{S_{cen}}$ for K must result in a disproportionate change of $P_{S_i}$ for both ions, since $P_{S_{cap}}$ reflects a different proportion of the $P_{S_i}$ for each. From our evaluation of these proportions in the control state, we may predict how much the ratio of $P_{S_{Rb}}$ to $P_{S_K}$ should change for a given increase or decrease of capillary surface and estimate the magnitude of the errors resulting from use of $P_{S_{Rb}}$ or $P_{S_K}$ as an index of $P_{S_{cap}}$. K in experiments such as those listed in Table 4 of this paper.

**Effects of Local Anesthetics on Steady-State K and Rb Exchange**

Local anesthetics reduce resting permeability to K in nerve and muscle (18). It has been proposed for frog muscle that cocaine blocks a membrane pathway accessible only to K and has little effect on another pathway through which both K and Rb move with equal mobility (31, 32). In our experiments with the dog gracilis, procaine and cocaine reduced both $P_{S_{Rb}}$ and $P_{S_K}$ and brought them closer together, but in no experiment did they become equal. Application to our data of the same analysis used in the previous section, assuming control $\alpha = 0.40$ and $R_{cell}$ to be unchanged, shows that cell membrane resistance to both ions was raised but that the effect was somewhat greater for K (Table 7). Averaging only the first drug addition in each experiment, $R_{cell}$ increased 2.3-fold, $R_{cell}$ increased 1.6-fold, and $\alpha$ rose from 0.40 to 0.62. The action of these agents on mammalian skeletal muscle is therefore not precisely the same as has been reported for amphibian muscle, since it reduces permeability to both ions, albeit more to K.

**Influence of Vasomotor Changes on Steady-State Rb and K Transport**

If $P_{Scap}$ alone is altered by vasomotor control of the number of open capillaries, the magnitude of the change will be underestimated by measurements of either $P_{S_{Rb}}$ or $P_{S_{K}}$ (6, 7, 20) due to the influence of the cell membrane as a barrier. This may explain part of the difference (usually only qualitative) between such estimates of effective capillary surface and those based on measurement of capillary filtration coefficients, which presumably depend only on the capillary barrier (33, 34). A change in $P_{Scap}$ unaccompanied by any change of $P_{S_{cell}}$ for Rb or K must result in a disproportionate change of $P_{S_i}$ for the two ions, since $P_{Scap}$ reflects a different proportion of the $P_{S_i}$ for each. From our evaluation of these proportions in the control state, we may predict how much the ratio of $P_{S_{Rb}}$ to $P_{S_K}$ should change for a given increase or decrease of capillary surface and estimate the magnitude of the errors resulting from use of $P_{S_{Rb}}$ or $P_{S_K}$ as an index of $P_{Scap}$ in experiments such as those listed in Table 4 of this paper.

Table 8 illustrates predicted changes in $P_{S_{Rb}}$ and $P_{S_K}$ for a twofold change in $P_{Scap}$ in either direction with no change in cell membrane permeability to these ions. Control $P_{Scap}$ is set equal to 1.00, and other values are taken to be inversely proportional to the partition of resistances.
given in Table 5 (Eqs. 7a and 7b). For reduction in $P_{\text{cap}}$ (vasoconstriction), $P_{\text{Hb}}$ would underestimate the change by one-third, $P_{\text{K}}$ by one-sixth. The ratio of $P_{\text{Hb}}$ to $P_{\text{K}}$ would be increased by about 15%; this is close to that actually observed in three of the four experiments with norepinephrine in Table 4. With still larger decreases in $PS_{\text{cap}}$, the error of using $P_S$ as an estimate will tend to be smaller, because the capillary barrier represents an increasingly large fraction of the total resistance to transport. The magnitude of error for changes in this direction is not excessive compared to other possible measures of effective capillary surface. For increased $PS_{\text{cap}}$, however, the underestimates are more serious. Furthermore, if $P_{\text{cell}}$ does not increase with $PS_{\text{cap}}$, it comes to be the dominating factor in $P_S$ for either ion. Estimates of increases in capillary surface made on the basis of steady-state equilibration of $^{86}\text{Rb}$ or $^{42}\text{K}$ have generally been smaller than estimates made from capillary filtration coefficients or anatomical measurements. For example, we reported an increase of $P_{\text{Hb}}$ of

\begin{table}
\begin{center}
\begin{tabular}{|c|c|c|c|c|}
\hline
\textbf{Expt} & \textbf{Procedure} & $R_{\text{cell}} \text{Rb}$ & $R_{\text{cell}} \text{K}$ & $a$ \\
\hline
3 & Control & 0.210 & 0.084 & 0.40 \\
 & Procaine (1) & 2.0 & 2.8 & 1.4 \\
 & Procaine (2) & 4.1 & 8.7 & 2.1 \\
4 & Control & 0.162 & 0.065 & 0.40 \\
 & Procaine (1) & 1.2 & 2.0 & 1.6 \\
 & Procaine (2) & 1.6 & 3.1 & 2.0 \\
5 & Control & 0.175 & 0.070 & 0.40 \\
 & Procaine & 1.9 & 2.1 & 1.1 \\
6 & Control & 0.243 & 0.097 & 0.40 \\
 & Procaine & 1.4 & 2.3 & 1.7 \\
7 & Control & 0.140 & 0.056 & 0.40 \\
 & Procaine & 2.0 & 3.8 & 1.8 \\
8 & Control & 0.307 & 0.123 & 0.40 \\
 & Procaine & 0.73 & 1.4 & 1.9 \\
9 & Control & 0.347 & 0.139 & 0.40 \\
 & Procaine & 1.6 & 2.2 & 1.4 \\
 & Recovery & 1.3 & 1.8 & 1.3 \\
10 & Control & 0.342 & 0.137 & 0.40 \\
 & Procaine & 1.7 & 1.9 & 1.5 \\
11 & Control & 1.6 & 2.3 & 1.6 \\
\hline
\end{tabular}
\end{center}
\begin{tabular}{l}
\textbf{Mean} \\
\hline
\end{tabular}
\begin{tabular}{l}
\textbf{a} = 0.40 of changes during \\
first administration of drug only \\
\hline
\end{tabular}
\end{table}

Capillary resistance is assumed unchanged and the control value of $a = 0.40$. Changes produced by the drugs are given as multiples of the control value for that experiment.

\begin{table}
\begin{center}
\begin{tabular}{|c|c|c|c|}
\hline
\textbf{Control} & Vasoconstriction & Vasodilatation \\
\hline
\textbf{PS}_{\text{IK}} & 0.70 & 0.41 & 1.07 \\
\textbf{PS}_{\text{Hb}} & 1.00 & 0.50 & 2.00 \\
\textbf{PS}_{\text{cap}} & 2.30 & 2.30 & 2.30 \\
\textbf{PS}_{\text{Hb}} & 0.48 & 0.33 & 0.64 \\
\textbf{PS}_{\text{cell}} & 1.00 & 0.50 & 2.00 \\
\textbf{PS}_{\text{cap}} & 0.93 & 0.93 & 0.93 \\
\textbf{PS}_{\text{Hb}}/\textbf{PS}_{\text{IK}} & 0.70 & 0.80 & 0.60 \\
\hline
\textbf{AP}_{\text{IK}} & -50\% & +100\% \\
\textbf{AP}_{\text{Hb}} & -42\% & +52\% \\
\textbf{AP}_{\text{cell}} & -33\% & +33\% \\
\hline
\end{tabular}
\end{center}
\begin{tabular}{l}
Vasodilatation is assumed to double $PS_{\text{cell}}$ vasoconstriction to reduce it to half the control value. Control $PS_{\text{Hb},K} = 1.00$. See Eqs. 7a and 7b. \\
\end{tabular}
\end{table}
scarcely twofold in contracting mammalian skeletal muscle (20) (Table 4), while Kjell-mer found that the filtration coefficient was increased three-to fourfold (34).

Our data for contracting muscles disagree with our prediction that the ratio of $PS_{\text{Rb}}$ to $PS_{\text{K}}$ should fall when capillary surface is increased. Little change in this ratio is shown. It is possible that cell permeability to Rb and K was increased in proportion to the increase in capillary surface. This seems unlikely because the cell membranes were depolarized only twice per second—only a small percent of the time. A second possibility is that the geometry of the interstitial space limits the effective surface of cell membrane exposed to interchange with blood-borne tracer to that closely adjacent to open capillaries. Except immediately around the capillaries, the spaces between muscle cells are narrow clefts (Fig. 6). Since Rb and K are rapidly taken up from the interstitial fluid by the cells, the probability of a tracer ion migrating far from a capillary is small. For such substances the interstitium cannot be considered a well-mixed pool, despite their rapid diffusibility in it. Ziegler and Goresky (35) have recently shown that this is the case also for antipyrine and tritiated water in heart muscle, though the homogeneous compartment model holds reasonably well for solutes which do not penetrate cells so rapidly. This kind of geometric effect causes difficult problems for the general theory of blood-tissue interchange and for measurement of cell membrane exchange rates from arteriovenous differences and flow data; however, it has the fortunate result of allowing lumped $PS$ values for $^{86}$Rb and $^{42}$K to be used as indicators of changes in capillary surface under conditions in which cell membrane permeability is not changed.

Appendix

GEOMETRIC ANALYSIS OF INTERSTITIAL DIFFUSION

This analysis is similar to that of Blum (36) and is presented here to clarify our application of it. The terminology follows that of Blum except that we use $B$ and $b$ instead of $R$ and $r_n$, due to our previous use of $R$ for resistance. The following assumptions are made (Fig. 6). (1) $^{86}$Rb and $^{42}$K

![Diagram of an element of the diffusion model](http://circres.ahajournals.org/)

**FIGURE 6**

Diagram of an element of the diffusion model; a: End view. b: Side view. Cells of skeletal muscle are stippled. $L =$ capillary length, $b =$ capillary radius. $B$ is not half the distance between adjacent capillaries, but the effective distance from the center of the capillary to adjacent muscle cell membrane. $B = b =$ interstitial diffusion distance. For calculations, $L$ is taken to be 1 mm, $b$ 3.5μ, and $B$ between 5 and 50μ. The lower figure represents a diffusion distance from capillary wall to cell membrane of 1.5μ and is probably a fair estimate of this quantity. The other is the highest possible value for a tissue with intercapillary distances of 100μ.
diffuse from a central capillary into the interstitial fluid and from the interstitial fluid across the muscle cell membrane into a pool of 39K. (2) All capillaries are identical and perfused in parallel. (3) Radial concentration gradients within the capillary are negligible (37). (4) Longitudinal diffusion outside the capillary is negligible. (5) No material transfer occurs across the ends of the cylinder of interstitial fluid associated with each capillary. (6) Capillary lumen, interstitial fluid, and intramuscular fluid are in a steady state with respect to 86Rb and 42K. The last assumption is an acceptable approximation for 86Rb and 42K fluxes during the plateau of continuous-infusion experiments in skeletal muscle because of the very large pool of intracellular 39K with which these tracers mix. Specific activity of cell K rises very slowly, and back-diffusion remains small compared to tracer flux into the cells (see first section of Discussion).

Variables.—u, specific activity of 42K or 86Rb (count min⁻¹ mEq⁻¹ 38K); z, longitudinal distance along capillary; r, radial distance from center of capillary; u(z), capillary specific activity as a function of distance along capillary; uᵢ(r, z), specific activity of interstitial fluid as a function of radial and longitudinal dimensions; w₀, specific activity inside muscle cells.

Parameters.—b, capillary radius (cm); L, capillary length; B, radius of interstitial cylinder; N, number of capillaries per 100 grams of muscle; S, capillary surface = 2π bLN cm²/100 g. PS cap, permeability-surface area product for capillary wall (ml/[min × 100 g]), see text; Q_p, plasma flow (ml/[min × 100 g]), for a single capillary.

For the capillary, isotope brought into the disc by plasma flow equals isotope leaving by plasma flow and diffusion across capillary wall. (A1)

For the interstitial fluid, in the steady state only, the rate of increase of isotope in any infinitesimal volume equals zero. (A2)

In the steady state, once a stable configuration of specific activities is attained in the capillary and the interstitial space, the following conditions apply along the various boundaries:

The net rate at which isotope crosses the capillary wall equals the rate at which isotope crosses the muscle cell membrane. (A3)

Isotope arriving at the muscle cell membrane will then equal that crossing the muscle cell membrane. (A4)

At z = 0, u(z) = u₀ (A5)
At z = L, u(z) = u_v (A6)

Eq. A1 gives rise to the differential equation

\[
\frac{du(z)}{dz} = \frac{PS_{cap}}{Q_p L} [u(z) - u_i(b, z)].
\]

Eq. A2 is the steady-state, one-dimensional diffusion equation. Expressed in polar coordinates,

\[
\frac{\delta^2 u_i(r, z)}{\delta r^2} + \frac{1}{r} \frac{\delta u_i(r, z)}{\delta r} = 0.
\]

Boundary conditions (Eqs. A3 and A4) give rise to the following:

\[
PS_{cap} [u(z) - u_i(b, z)] = \rho (u_i(b, z) - u_0).
\]

Eq. A8 has the general solution

\[
u_i(r, z) = A_1 + A_2 \ln r,
\]

where A₁ and A₂ are constants. These may be expressed in terms of u(z) by substituting uᵢ(r, z) from Eq. A11 into Eqs. A9 and A10.

Consider a thin tissue disc, radial to the long axis of the capillary, of thickness dz. The area of capillary wall in this disc is Sdz/LN, the area of cell membrane is approximately 2πBdz. The following equations of material balance hold within the disc:

\[
-\frac{D[K]}{r} \left[ \frac{\delta u_i(r, z)}{\delta r} \right] = \frac{\delta w}{\delta z}.
\]

Now uᵢ(b, z) may be stated in terms of u(z) and the various parameters and substituted into Eq. A7:

\[
\frac{du(z)}{dz} = -\frac{PS_{cap}}{Q_p L} [u(z) - u_0] G,
\]

where

\[
G = \frac{2\pi LDN \rho}{2\pi LDN (\rho + [K]PS_{cap}) - PS_{cap} \rho \ln(b/B)}.
\]
Table 9

Calculated Interstitial Diffusion Resistance ($R_{ist}$)

<table>
<thead>
<tr>
<th>$D$ (cm $^2$/min)</th>
<th>5</th>
<th>10</th>
<th>20</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^{-6}$</td>
<td>0.018</td>
<td>0.052</td>
<td>0.087</td>
<td>0.133</td>
</tr>
<tr>
<td>$10^{-4}$</td>
<td>0.002</td>
<td>0.005</td>
<td>0.009</td>
<td>0.013</td>
</tr>
<tr>
<td>$10^{-4}$</td>
<td>0.0002</td>
<td>0.0005</td>
<td>0.0009</td>
<td>0.0013</td>
</tr>
<tr>
<td>$10^{-3}$</td>
<td>0.00002</td>
<td>0.00005</td>
<td>0.00009</td>
<td>0.00013</td>
</tr>
</tbody>
</table>

$b =$ capillary radius; $L =$ capillary length; $N =$ number of capillaries per 100 grams of muscle; $B =$ radius of interstitial cylinder; $D =$ free diffusion coefficient.

$R_{ist} = 1/PS_{ist} = -\ln (b/B)/2\pi LDN, b = 3.5 \times 10^{-4}$ cm, $L = 10^{-1}$ cm; $N = 32 \times 10^8$ caps/100 g.

Eq. A14 may be integrated directly applying boundary conditions (Eqs. A5 and A6).

$$u_v = u_0 + (u_a - u_0) \exp \left( -\frac{PS_{cap} G}{Q_p} \right). \quad (A16)$$

Since $u_v/u_a = V$ and $u_0/u_a = T$ as defined in the main part of this paper, this may be rewritten

$$V = T + (1 - T) \exp \left( -\frac{PS_{cap} G}{Q_p} \right) \quad (A17)$$

or

$$PS_{cap} = -\frac{Q_p}{G} \ln [(V - T)/(1 - T)]. \quad (A18)$$

According to previous definitions,

$$PS_i = -Q_p \ln [(V - T)/(1 - T)] \quad (A19)$$

and

$$PS_{cell} = \rho/[K], \quad (A20)$$

from which it follows (substituting $G$ from Eq. A15) that

$$\frac{1}{PS_i} = \frac{1}{PS_{cap}} + \frac{1}{2\pi LDN} + \frac{1}{PS_{cell}}. \quad (A21)$$

This is equivalent to Eq. 4b in the text, with

$$\frac{1}{PS_{ist}} = -\ln (b/B) \frac{1}{2\pi LDN}. \quad (A22)$$

The contribution of the interstitial fluid to overall resistance to transport can be evaluated by substituting appropriate values in Eq. A22, and comparing $1/PS_{ist}$ (or $R_{ist}$) with values of $R_{tot}$, $R_{exp}$, and $R_{cel}$ tabulated in the main part of this paper. The geometric parameters used are given in the legend to Figure 6. For $N$ we have taken $32 \times 10^8$ capillaries/100 g, corresponding to an effective surface area of $7 \times 10^8$ cm$^2$ (8), or an intercapillary distance of about $40 \mu$. Table 9 lists calculated $R_{ist}$ (min/cm$^3$) for $D = 10^{-6}, 10^{-4}, 10^{-3}$ cm$^2$/min. The free diffusion coefficient for Na in water at $37^\circ$C is approximately $10^{-3}$ cm$^2$/min and for Rb and K, $1.4 \times 10^{-3}$ cm$^2$/min. Reported values for diffusibility of these ions in the interstitium of mammalian skeletal muscles are about half the free values (29). Comparison of the predicted $R_{ist}$'s with measured values of $1/PS_{cap}$ for Rb, K, and Na in Table 1 and with $R_{tot}$, $R_{exp}$, and $R_{cel}$ for Rb and K in Tables 5 and 6 (the units are the same as in Table 9) shows that $R_{ist}$ must be less than 5% of the measured $R$'s for these ions unless their $D$ in the interstitium of mammalian skeletal muscle is much less than $1/100th$ of the free value or interstitial diffusion distances substantially exceed $20\mu$.

References

BARRIERS TO BLOOD-TISSUE TRANSPORT

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R. M. SHEEHAN and E. M. RENKIN

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