Dynamics and Consequences of Potassium Shifts in Skeletal Muscle and Heart During Exercise

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Sejersted, Ole M., and Gisela Sjøgaard. Dynamics and Consequences of Potassium Shifts in Skeletal Muscle and Heart During Exercise. Physiol Rev 80: 1411–1481, 2000.—Since it became clear that K⁺ shifts with exercise are extensive and can cause more than a doubling of the extracellular [K⁺] (\([K^+]_e\)) as reviewed here, it has been suggested that these shifts may cause fatigue through the effect on muscle excitability and action potentials (AP). The cause of the K⁺ shifts is a transient or long-lasting mismatch between outward repolarizing K⁺ currents and K⁺ influx carried by the Na⁺-K⁺ pump. Several factors modify the effect of raised \([K^+]_e\) during exercise on membrane potential (\(E_m\)) and force production. 1) Membrane conductance to K⁺ is variable and controlled by various K⁺ channels. Low relative K⁺ conductance will reduce the contribution of [K⁺]_e to the \(E_m\). In addition, high Cl⁻
conductance may stabilize the $E_m$ during brief periods of large $K^+$ shifts. 2) The $Na^+\text{-}K^+$ pump contributes with a hyperpolarizing current. 3) Cell swelling accompanies muscle contractions especially in fast-twitch muscle, although little in the heart. This will contribute considerably to the lowering of intracellular [K$^+$] ([K$^+$]$_v$) and will attenuate the exercise-induced rise of intracellular [Na$^+$] ([Na$^+$]$_v$). 4) The rise of [Na$^+$]$_c$ is sufficient to activate the Na$^+\text{-}K^+$ pump to completely compensate increased K$^+$ release in the heart, yet not in skeletal muscle. In skeletal muscle there is strong evidence for control of pump activity not only through hormones, but through a hitherto unidentified mechanism. 5) Ionic shifts within the skeletal muscle t tubules and in the heart in extracellular clefts may markedly affect excitation-contraction coupling. 6) Age and state of training together with nutritional state modify muscle K$^+$ content and the abundance of Na$^+\text{-}K^+$ pumps. We conclude that despite modifying factors coming into play during muscle activity, the K$^+$ shifts with high-intensity exercise may contribute substantially to fatigue in skeletal muscle, whereas in the heart, except during ischemia, the K$^+$ balance is controlled much more effectively.

I. INTRODUCTION

Thanks to the pioneering work by W. O. Fenn during the 1930s, it was convincingly demonstrated that K$^+$ was lost from skeletal muscles during repeated contractions (194). Frog, rat, and cat muscles were studied during direct and indirect stimulation as well as during voluntary contractions. All showed the same general pattern of K$^+$ loss (192, 193). Simultaneous changes of intracellular Na$^+$ were also described, and both K$^+$ and Na$^+$ changes were monitored during recovery (195).

However, a number of questions were unresolved, such as which mechanisms on the sarcolemmal level cause these ion shifts, i.e., change in distribution between compartments? What is the magnitude of the changes in terms of content or concentration in the various spaces (intracellular, interstitial, and vascular spaces)? How are the K$^+$ fluxes regulated during muscle activity as well as during recovery? Muscle activity refers to the generation of action potentials in the muscle that may lead to development of force and/or shortening. Special attention must in this context be paid to the 1997 Danish Nobel prize winner in Chemistry J. C. Skou, who in 1957 presented convincing evidence for the existence of an enzyme system involved in the active extrusion of Na$^+$ from nerve fibers (603). Through a series of systematic studies (604–606) he was able to ascertain the molecular basis for active membrane transport systems and later reviewed the enzymatic basis for active transport of Na$^+$ and K$^+$ across the cell membrane in Physiological Reviews (607). Today this enzyme system is commonly referred to as the Na$^+\text{-}K^+$ pump and has been the focus of attention of several more recent reviews (99, 103, 112).

Another more recent finding that sparked new interest in the role of K$^+$ for skeletal muscle function was the very large fluctuations of plasma [K$^+$] witnessed during and immediately after exercise (189, 206, 207, 262, 467, 658). In Figure 1, we have reproduced data from two different experiments, one with dynamic bicycle exercise, and the other with static hand-grip contraction. In both cases very rapid and large increases were observed in venous plasma [K$^+$] during exercise. When contractile activity ceased, venous [K$^+$] rapidly fell to below normal resting value.

The ultimate function of muscle tissue is its ability to develop mechanical tension. A prerequisite for tension development, however, is the membrane excitability, i.e., its ability to generate and propagate action potentials (AP). This unique feature, which muscle cells have in common with nerve fibers, depends on [K$^+$] gradients across the membranes. From the large changes shown in Figure 1, it has been suggested that the K$^+$ shifts might affect excitability and contractile force.

![Figure 1. Effluent venous plasma K$^+$ concentration ([K$^+$]$_v$) monitored in the femoral vein in one representative experiment by a K$^+$-selective electrode during bicycling at 140% of maximum O$_2$ consumption (V$_{O2\text{max}}$) (solid line without symbols) and in blood samples from the antecubital vein during static handgrip contraction at 30% of maximal voluntary contraction (open circles; means ± SE). Muscle activity started at time 0 and ceased at times indicated by arrows. The transient, postexercise reduction of [K$^+$]$_c$ to below normal resting values lasted for 10–15 min. [Data from Vøllestad et al. (658) and Fallentin et al. (189).]]
One of the most crucial questions that is examined in detail in this review is the significance of K⁺ translocations for muscle performance and fatigue. Scrutinizing the variability of responses in different species or muscle fiber types may reveal common mechanisms. It was not until the 1960s that the different fiber types became a focus of research regarding ionic composition at rest and with exercise (616, 617, 619). At rest, important differences were revealed between slow-twitch and fast-twitch muscles regarding electrolytes and membrane potential \( (E_{m}) \) (84, 95, 96, 619). It is important to conduct a detailed analysis of such differences if one is to properly understand and predict the responses in humans during voluntary contractions connected with training and fatigue.

K⁺ shifts may also be important for cardiac function (385). First of all, variations in beating frequency will cause K⁺ shifts within the heart as will exposure of the heart to catecholamines. It is of special interest to compare the heart with skeletal muscle, because the normal heart is capable of minimizing K⁺ perturbations much more effectively than skeletal muscle (564). However, in this review it would be unrealistic to cover the vast literature regarding the role of K⁺ in cardiac excitability, especially since ischemia causes rapid and large perturbations of cardiac K⁺ homeostasis, which has been the focus of intensive research (11, 121, 230, 289, 357, 358, 365, 533, 581, 680, 683, 697, 712). We therefore restrict references to the cardiac literature to those occasions when we want to make comparisons with skeletal muscle that may help in clarifying important control mechanisms. The second point to make is that the rise in plasma K⁺ that occurs with exercise will of course affect other excitable tissues, especially the heart.

The development of new analytical tools provided for more insight into the subtle electrolyte fluxes. One line of development was the introduction of ion-selective electrodes that allowed continuous measurements both on the microscopic level inside living cells and in the extracellular spaces (204, 248, 254, 290, 296, 396, 424, 425). Methods for analysis of ion concentrations are dealt with in section II. Another line of development was the biochemical analysis and quantification of membrane proteins, e.g., the Na⁺-K⁺ pump (99). The perturbations to which various preparations have been exposed are often nonphysiological, and often only a single variable is manipulated at a time. We certainly learn from these manipulations, but to fully understand the complex ion shifts and their effects on muscle and heart function, all this information must be integrated and tested in vivo.

A large amount of literature is available which deals with the translocation of K⁺ in muscle tissue that is active, and a variety of approaches have been used. This is also the reason why many different terminologies are being used. When studying a single muscle cell in bathing solution, it is necessary to distinguish between intracellular \([K⁺]_{i}\) and \([K⁺]_{o}\) outside the cell, that is, the bathing solution or the extracellular concentration on the surface of the cell membrane. The nomenclatures outside and extracellular are used interchangeably in the literature and suffixes such as “o” (81, 588, 613), “out” (120, 585), or “e” (294, 296) are also used. When dealing with the in vivo situation, the extracellular space is composed of two distinctly separated spaces: the interstitial space and the intravascular space, and one might also add the intralymphatic space. This has led to confusion because the suffix “i” may denote any of the different spaces inside the various compartments: interstitial (125), intravascular (i.e., including capillary and lymph) or just inside-the-capillary (214), but most often intracellular (122, 496, 585, 595, 616). Sometimes “i” simply stands for intramuscular (123). Interstitial may also be abbreviated “is” (122) or “isf” (270) in contrast to “icf” for intracellular versus “ecf” for extracellular (84), the latter of which corresponds to “isf.” Therefore, in this review we have totally omitted the suffix “i” and instead we introduce “c” to denote cellular or cytosolic concentration or content. As far as the spaces outside the cell are concerned, we use “s” to specify surface concentrations, which then correspond to the interstitial concentration, and “e” for the gross extracellular space, including interstitial as well as vascular compartments. Further details of terminology and definitions are dealt with in the model description in section II.

II. DISTRIBUTION SPACES FOR POTASSIUM

A. Methods for Measuring K⁺

Sampling site, sampling technique, and analytical technique must all be considered when judging a measured value of K⁺ content or concentration. As an example of the importance of the sampling site and technique, Farber et al. (191) noted more than 40 years ago that the standard technique of opening and closing the fist to fill the veins so as to facilitate puncturing consistently caused an artifactual increase of \([K⁺]_{s}\) (240, 334, 438a, 450). Furthermore, some investigators have inserted K⁺-selective mini-electrodes directly into the muscle tissue as discussed in section VIII (296, 297). It should be noted that even a minor mechanical injury of
the muscle cells will lead to vast overestimation of \([\text{K}^+]_s\) when the intracellular \([\text{K}^+]\) leaks out. Such injury is especially likely to occur with electrodes or catheters inserted into the exercising muscle. Table 1 summarizes the various analytical techniques that have been used. Of special interest for this review are the techniques for measuring or estimating the \([\text{K}^+]\) in plasma \((\text{[K}^+]_p)\), intracellular \([\text{K}^+]\) \((\text{[K}^+]_c)\), and \([\text{K}^+]_s\) and possibly \([\text{K}^+]\) in the t tubules and in the subsarcolemmal space.

Ion-selective electrodes have now largely replaced flame photometry in the laboratory. Such electrodes can also be manufactured for intracellular and intravascular use (254, 396). These electrodes measure ion activities which by definition are lower than concentration measured by flame photometry (707). Original observations also indicated that intracellular ion activities were lower than expected compared with \([\text{K}^+]\) in simple solution (97, 396). This led the authors to point out that some \([\text{K}^+]\) may be bound or compartmentalized in the cytoplasm. The \(39\text{K}\)-NMR technique substantiated that compartmentalization was likely, and it was shown that under certain conditions up to 15% of \([\text{K}^+]\) may be sequestered within an intracellular compartment (3). However, because similar differences are reported even in blood, binding of \([\text{K}^+]\) cannot be excluded in plasma and serum samples (204, 707). In recent years, ion-selective fluorescent dyes in combination with ordinary or confocal microscopy have largely replaced ion-selective electrodes in cellular work. A fluorescent probe for \([\text{K}^+]\) was recently proven to be suited for intracellular application (471). With regard to the role of \([\text{K}^+]\) in muscle fatigue, the concentration in the t-tubular system is probably important as discussed in section IV D. As yet, few studies have addressed this directly. One attempt to quantify electrolyte concentration was made by electron probe X-ray microanalysis in fatigued muscles with vacuolated t tubules (236, 610). Other studies have based estimates on changes in intra-t-tubular electrolyte concentration on indirect evidence or mathematical modeling that takes into account the narrow and tortuous diffusion path for \([\text{K}^+]\) in the lumen of the t tubules (14, 222, 386, 387), as will be further discussed in section IV D.

Radioactive isotopes of \([\text{K}^+]\) or its congener \(\text{Rb}^+\) have been extensively used to measure unidirectional fluxes between compartments and were instrumental in the early studies (299). The use of isotopes is still the preferred method of choice for this purpose. This has not been illustrated in Table 1 but is discussed in section VIII A. It should also be pointed out that some methods are not ethically applicable to humans (e.g., the use of some isotopes), although combining such methods in animal studies with those acceptable in humans does provide reliable estimates.

B. Distribution of \([\text{K}^+]\) in the Body

The total body \([\text{K}^+]\) content in normal human adults ranges from \(\sim 2,000\) to \(\sim 4,000\) mmol (58, 448). The total quantity depends on body weight and body fat content, and values in the order of 60 mmol/kg fat free body mass, ranging from 50 to 70 mmol/kg, have been reported (283, 432). The distribution of \([\text{K}^+]\) within the various body tissues is highly variable, which is true in terms of concentration as well as content. Around 2% of total \([\text{K}^+]\) is located in the extracellular space with normal plasma concentrations at rest ranging from 3.5 to 5.3 mM (707), while 98% is located intracellularly with concentrations in muscle tissues around 160 mM. The largest fraction of \([\text{K}^+]\) (corresponding to 75%) is located in the skeletal muscles,

### Table 1. Examples of methods used to determine \([\text{K}^+]\) in various tissue compartments (by reference number)

<table>
<thead>
<tr>
<th>Tissue Compartment and Specimen for Measurement/Recording Site</th>
<th>Methods of Measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ion-selective electrodes</td>
</tr>
<tr>
<td>Plasma</td>
<td>Direct 125, 229, 254, 294, 295, 424</td>
</tr>
<tr>
<td></td>
<td>Sample 74, 204, 324, 380, 422, 707</td>
</tr>
<tr>
<td>Lymph</td>
<td></td>
</tr>
<tr>
<td>Interstitial fluid</td>
<td>Direct 26, 27, 229</td>
</tr>
<tr>
<td></td>
<td>Wicks/capsules 240, 334, 438a</td>
</tr>
<tr>
<td></td>
<td>Microdialysis 414, 394, 396</td>
</tr>
<tr>
<td>In situ</td>
<td></td>
</tr>
<tr>
<td>Whole muscle</td>
<td>Direct 143, 329, 394, 396</td>
</tr>
<tr>
<td>Homogenate</td>
<td>44, 123, 412, 413, 592, 616</td>
</tr>
<tr>
<td>Whole body</td>
<td>In situ 8, 44, 46, 104, 122, 195, 412, 413, 616</td>
</tr>
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which constitute ~40% of body weight or ~30 kg wet wt, while heart muscle weighing around 0.3 kg accounts for only ~1% of total body K\(^+\). The erythrocytes carry ~7% of total body K\(^+\) and 8% is located in the bones, while the rest is located in other tissues. The muscle K\(^+\) content is 75–105 mmol/kg wet wt, depending on the fat content of the muscles. In obese people, if correction is not made for fat content, muscle K\(^+\) content may be considerably reduced, as the K\(^+\) content per kilogram body weight (383). In the following muscle K\(^+\) content refers to fat-free tissue and comprises the sum of K\(^+\) content in all muscle compartments (K\(^+_m\)). Also, in a number of species, different muscle fiber populations show differences in K\(^+_m\) as well as in intracellular [K\(^+\)], with the slow-twitch muscles having lower values than the fast-twitch muscle (84, 95, 619). However, species differences do exist, and in humans, the two main muscle fiber types did not differ in this respect (589). The loss of K\(^+\) from the body mainly occurs in feces and urine and is in general <25 mmol/day (503). The K\(^+\) loss in sweat is usually small but can approach a few percent of body content per day during very heavy sweating. The resting concentrations in the different body compartments are maintained within narrow limits by regulatory mechanisms of the cell surface membranes.

C. An Integrated Model of K\(^+\) Distribution and Flux Pathways in the Body

The complexity of the processes involved in K\(^+\) shifts in the body with exercise along with the relatively large number of compartments involved prompted us to create a simple model to make it easier to understand and analyze the kinetics and magnitude of the shifts. When there is a change in K\(^+\) flux across the endothelium or sarcolemma, or a volume change of the muscle tissue, K\(^+\) concentrations in several compartments will be affected. Figure 2 shows that in muscle there are three main compartments among which K\(^+\) moves: the intracellular muscle cell volume, the interstitial space, and the plasma volume of the microcirculation, which have been designated with the letters c, s, and p, respectively. We consider the volume of lymphatics (l) as part of the interstitial space. In addition circulating red blood cells (r) in the capillaries represent a possible exchange volume. The exchange of K\(^+\) between plasma and the red blood cells is treated separately in section viID. At rest, the intracellular compartment contains 85–91% of the total skeletal muscle water content, whereas 9–15% is located extracellularly (5, 592, 600). The corresponding values for the rat heart were reported as 73% and as high as 27%, respectively. The capillary volume in skeletal muscle is only ~1% of the tissue wet weight and thus takes up ~10% of the extracellular water volume (147). Again, in the rat heart, the capillary volume seems to be fairly large, amounting to almost 6% of the tissue wet weight or ~30% of the extracellular water volume. In many circumstances, [K\(^+\)] in the interstitial space and in the plasma are similar and may be regarded as the common extracellular concentration, denoted “e.” However, the interstitial space is large enough to have a considerable capacity for accumulating K\(^+\) in which case a change of K\(^+\) flux across the sarclemma will only be evident in the venous effluent with an attenuation as described below. Therefore, K\(^+\) events at the sarclemma must be considered separate from the exchange of K\(^+\) between the muscle tissue and the general circulation. Correspondingly, quantification of K\(^+\) release from the cells (Fig. 2) based on measurements of [K\(^+\)]\(_p\) together with muscle blood flow call for careful assumptions, the validity of which may not always be justified.

There is no standard nomenclature for the fluxes between the different compartments in the muscle tissue, and Tables 2 and 3 have been constructed as an attempt

![Figure 2](http://physrev.physiology.org/)
to establish an unequivocal terminology. The following model is to a large extent based on the models published by Hallén and Sejersted (254) and Sjøgaard (594), but has now been extended and presented more systematically. Figure 3 shows in more detail the various flux expressions (J) and abbreviations used for volume, K⁺ content, and concentration in the various compartments. In addition, [K⁺]_{v,mix} and [K⁺]_{p,mix} are used for the concentration of K⁺ in mixed venous blood and at unspecified sampling sites, respectively.

### Table 2. Symbols and descriptions for variables in a model of muscle K⁺ fluxes

<table>
<thead>
<tr>
<th>Symbols</th>
<th>Description</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>( V_c )</td>
<td>Cellular fluid volume (intracellular)</td>
<td>l</td>
</tr>
<tr>
<td>( K_c^+ )</td>
<td>Cellular K⁺ content (intracellular)</td>
<td>mmol</td>
</tr>
<tr>
<td>([K⁺]_{c} )</td>
<td>Cellular K⁺ concentration (( K_c^+ \cdot V_c^{-1} )) (intracellular)</td>
<td>mM</td>
</tr>
<tr>
<td>( dV_c \cdot dt^{-1} )</td>
<td>Rate of change of cellular fluid volume</td>
<td>l/s</td>
</tr>
<tr>
<td>( dK_c^+ \cdot dt^{-1} )</td>
<td>If &gt;0, net uptake rate by the muscle cells = rate of intracellular K⁺ accumulation</td>
<td>mmol/s</td>
</tr>
<tr>
<td>( d[K⁺]_{c} \cdot dt^{-1} )</td>
<td>If &lt;0, net release rate from muscle cells = rate of intracellular K⁺ dissipation</td>
<td>mmol/s</td>
</tr>
<tr>
<td>K⁺ uptake</td>
<td>Accumulated cellular K⁺ uptake over time</td>
<td>mmol</td>
</tr>
<tr>
<td>K⁺ release</td>
<td>Accumulated cellular K⁺ release over time</td>
<td>mmol</td>
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</tbody>
</table>

### Interstitial space (s)

<table>
<thead>
<tr>
<th>Symbols</th>
<th>Description</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>( V_s )</td>
<td>Interstitial fluid volume</td>
<td>l</td>
</tr>
<tr>
<td>( K_s^+ )</td>
<td>Amount of K⁺ in the interstitial fluid</td>
<td>mmol</td>
</tr>
<tr>
<td>([K⁺]_{s} )</td>
<td>Interstitial K⁺ concentration (( K_s^+ \cdot V_s^{-1} ))</td>
<td>mM</td>
</tr>
<tr>
<td>( dV_s \cdot dt^{-1} )</td>
<td>Interstitial accumulation or K⁺ dissipation rate</td>
<td>l/s</td>
</tr>
<tr>
<td>( d[K⁺]_{s} \cdot dt^{-1} )</td>
<td>Rate of interstitial K⁺ concentration change</td>
<td>mM/s</td>
</tr>
</tbody>
</table>

### Lymphatics (l)

<table>
<thead>
<tr>
<th>Symbols</th>
<th>Description</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>( V_l )</td>
<td>Fluid volume inside lymph vessels within the muscle</td>
<td></td>
</tr>
<tr>
<td>([K⁺]_{l} )</td>
<td>Lymph fluid K⁺ concentration</td>
<td></td>
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</table>

### Intracapillary plasma (p)

<table>
<thead>
<tr>
<th>Symbols</th>
<th>Description</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>( V_p )</td>
<td>Intracapillary plasma fluid volume (intravascular fluid)</td>
<td>l</td>
</tr>
<tr>
<td>( K_p^+ )</td>
<td>Amount of K⁺ in the intravascular fluid</td>
<td>mmol</td>
</tr>
<tr>
<td>([K⁺]_{p} )</td>
<td>Intracapillary plasma K⁺ concentration</td>
<td>mM</td>
</tr>
<tr>
<td>( V_e )</td>
<td>Intramuscular extracellular volume (( V_s + V_p + V_l ))</td>
<td>l</td>
</tr>
<tr>
<td>( dK_p^+ \cdot dt^{-1} )</td>
<td>K⁺ accumulation or dissipation rate in the intravascular</td>
<td>mmol/s</td>
</tr>
<tr>
<td>( d[K⁺]_{p} \cdot dt^{-1} )</td>
<td>Rate of intravascular K⁺ concentration change</td>
<td>mM/s</td>
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</table>

### Red blood cells (r)

<table>
<thead>
<tr>
<th>Symbols</th>
<th>Description</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>( K_r^+ )</td>
<td>Intracellular K⁺ content of red blood cells</td>
<td>mmol/l cells</td>
</tr>
<tr>
<td>([K⁺]_{r} )</td>
<td>Intracellular red blood cell K⁺ concentration</td>
<td>mM</td>
</tr>
</tbody>
</table>

### Artery (a)

<table>
<thead>
<tr>
<th>Symbols</th>
<th>Description</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>([K⁺]_{a} )</td>
<td>Arterial plasma K⁺ concentration</td>
<td>mM</td>
</tr>
</tbody>
</table>

### Vein (v)

<table>
<thead>
<tr>
<th>Symbols</th>
<th>Description</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>([K⁺]_{v} )</td>
<td>Effluent venous plasma K⁺ concentration</td>
<td>mM</td>
</tr>
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</table>

### General circulation

<table>
<thead>
<tr>
<th>Symbols</th>
<th>Description</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>([K⁺]_{v,mix} )</td>
<td>Mixed venous plasma K⁺ concentration</td>
<td>mM</td>
</tr>
<tr>
<td>([K⁺]_{p,mix} )</td>
<td>Plasma K⁺ concentration, unspecified sampling site</td>
<td>mM</td>
</tr>
</tbody>
</table>

### Whole muscle

<table>
<thead>
<tr>
<th>Symbols</th>
<th>Description</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>( K_m^+ )</td>
<td>Whole muscle K⁺ content</td>
<td>mmol/kg (dry or wet weight)</td>
</tr>
<tr>
<td>K⁺ gain rate</td>
<td>Net uptake rate of K⁺ by muscle (( J_{K_a,in} - J_{K_v,out} - J_{K_l,out} &gt; 0 ); see Table 3 for definitions)</td>
<td>mmol/s</td>
</tr>
<tr>
<td>K⁺ loss rate</td>
<td>Net loss rate of K⁺ from muscle (( J_{K_a,in} - J_{K_v,out} - J_{K_l,out} &lt; 0 ); see Table 3 for definitions)</td>
<td>mmol/s</td>
</tr>
<tr>
<td>K⁺ gain</td>
<td>Accumulated gain over time</td>
<td>mmol</td>
</tr>
<tr>
<td>K⁺ loss</td>
<td>Accumulated loss over time</td>
<td>mmol</td>
</tr>
</tbody>
</table>
For any compartment, the terms $K^+$ accumulation rate or $K^+$ dissipation rate are used for net changes in $K^+$ content (Table 2). At the cell membrane level, the term $K^+$ release rate means the normal net $K^+$ flux through various channels. In the model, the release rate ($J_{Ksc}$) will be negative for an outward repolarizing $K^+$ current (Table 3). This nomenclature contrasts with the convention of electrophysiology today where inward currents are negative. However, we wanted to maintain $K^+$ gain by the whole muscle as a positive value, and for the sake of consistency, inward currents are therefore positive.

Normally, the driving force for $K^+$ is directed out of the cell, resulting in an outward electrodiffusion of $K^+$. This is normally opposed by $K^+$ uptake mediated by the ATP-requiring Na$^+$-K$^+$ pump. Under certain circumstances discussed below, the net electrochemical driving force for $K^+$ may be directed into the cell, which means that there may be an inward $K^+$ flux through channels, i.e., a $K^+$ uptake in addition to that carried by the Na$^+$-K$^+$ pump (Table 2). Figure 3 and Table 3 also refer to $K^+$ transport through separate transporters, which may be important but which have been studied very little in muscle tissue. They may comprise the Na$^+$-K$^+$-2Cl$^-$ transporter and possibly a $K^+$/H$^+$ exchanger (see sect. uD).

Two terms, gain and loss, that are used in this review will need some clarification. When the net diffusion of $K^+$ across the capillary endothelium is directed from the interstitium into the capillary plasma, it is referred to as net $K^+$ efflux (which is a rate), whereas a net influx means that $K^+$ moves from the capillaries into the interstitium. The whole muscle will gain $K^+$ at a certain gain rate when a net amount of $K^+$ is transferred from the general circulation into the muscle, whereas the muscle tissue loses $K^+$ when the net movement of $K^+$ is in the opposite direction. It is important to take into consideration that the muscle tissue is supplied with $K^+$ from the arterial plasma ($K^+$ input rate) and that $K^+$ comes out of the muscle through both lymph and venous plasma. The sum of these two output rates is then the total $K^+$ output, with $K^+$ loss rate or $K^+$ gain rate being the absolute difference between the input and total output of $K^+$.

The amount of $K^+$ lost from one muscle or from the heart will distribute first in the plasma, then in the extracellular space of various organs, and finally $K^+$ will be

<table>
<thead>
<tr>
<th>Symbols</th>
<th>Definitions</th>
<th>Units</th>
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<tbody>
<tr>
<td>$E_m$</td>
<td>Membrane potential</td>
<td>mV</td>
</tr>
<tr>
<td>$E_K$</td>
<td>Equilibrium potential for $K^+$</td>
<td>mV</td>
</tr>
<tr>
<td>$J_{Ksc}$</td>
<td>$K^+$ diffusion rate through $K^+$ channels (outward &lt; 0; inward &gt; 0)</td>
<td>mmol/s</td>
</tr>
<tr>
<td>$J_{Na-K}$</td>
<td>Active $K^+$ pump rate through the Na$^+$-K$^+$ pump (always inward &gt; 0)</td>
<td>mmol/s</td>
</tr>
<tr>
<td>$J_{Ktransport}$</td>
<td>Rate of fluid transfer between intracellular volume and interstitial space (outward &lt; 0; inward &gt; 0)</td>
<td>l/s</td>
</tr>
<tr>
<td>$J_{Kps}$</td>
<td>Diffusion rate of $K^+$ through the capillary wall</td>
<td>mmol/s</td>
</tr>
<tr>
<td>$J_{Fsc}$</td>
<td>Rate of fluid transfer across the endothelium according to Starling forces</td>
<td>l/s</td>
</tr>
<tr>
<td>$J_{K_{l, out}}$</td>
<td>Lymph $K^+$ output rate ($[K^+]<em>{ls} \cdot J</em>{F_{l, out}}$)</td>
<td>mmol/s</td>
</tr>
<tr>
<td>$J_{F_{l, out}}$</td>
<td>Rate of lymph production</td>
<td>l/s</td>
</tr>
<tr>
<td>$J_{K_{v, out}}$</td>
<td>Venous $K^+$ output rate ($[K^+]<em>{v} \cdot J</em>{F_{v, out}}$)</td>
<td>mmol/s</td>
</tr>
<tr>
<td>$J_{F_{v, out}}$</td>
<td>Venous plasma fluid flow</td>
<td>l/s</td>
</tr>
<tr>
<td>$J_{K_{a, in}}$</td>
<td>Arterial $K^+$ input rate ($[K^+]<em>{a} \cdot J</em>{F_{a, in}}$)</td>
<td>mmol/s</td>
</tr>
<tr>
<td>$J_{F_{a, in}}$</td>
<td>Arterial plasma fluid flow</td>
<td>l/s</td>
</tr>
</tbody>
</table>

* Not included in flux equations. † $K^+$ carried by red blood cells not included.
taken up into cells of other muscles and other tissues. We have chosen to name this movement of K\textsuperscript{1} redistribution. The rate of redistribution will depend on many factors that are included in standard pharmacokinetic models. For the sake of simplicity, it is assumed that there are two accessible distribution volumes outside the working muscle, namely, the extra- and intracellular fluid volumes of other tissues. Some authors only take the plasma volume into account, for instance when the effect of hemocoagulation on arterial [K\textsuperscript{1}] (\([\text{K}^{1}]_{a}\)) is considered (239). This is clearly wrong since with the exception of the capillaries of the central nervous system K\textsuperscript{1} is rapidly equilibrated with the interstitial fluid volume of remote tissues that are adequately perfused. It is important that the rate of mixing within the extracellular fluid space is dependent on the flow rate through these tissues. For instance, with high-intensity exercise, flow through the splanchnic area may be reduced, and mixing in this area will then be slowed (251). It is of note that this mixing may still be considered fast relative to redistribution into other cells. The rate of intracellular accumulation is governed by the balance between K\textsuperscript{1} release and uptake rates across the cell membranes and, as will be discussed in section viA2, membrane conductance to K\textsuperscript{1} varies with the [K\textsuperscript{1}]\textsubscript{i}. Hence, four factors, tissue blood flow, the [K\textsuperscript{1}] gradient, membrane conductance, and activity of the Na\textsuperscript{+}-K\textsuperscript{1} pump, will together be major determinants of the rate of gain of K\textsuperscript{1} by cells in the remote tissues. Control mechanisms of the Na\textsuperscript{+}-K\textsuperscript{1} pump and K\textsuperscript{1} uptake mechanisms are treated in more detail in section III.B.

One of the aims of this review is to assess the magnitude of the fluxes illustrated in Figures 2 and 3 and described in Table 4, to which end various approaches have been taken. Radioisotopes have been widely used in all kinds of models, ranging from single cells, isolated muscles, muscle strips, perfused muscles, Langendorff heart preparations, and also intact organs in situ. Basically, because \(J_{\text{Na,K pump}} + J_{\text{Ksc}} = \frac{\text{d}[K^1]}{\text{d}t}\), one of these three parameters can be calculated on the basis of measurements of the two others. Significantly, when using \(^{42}\text{K}^1\), not only the net shift of K\textsuperscript{1} from one compartment to the other will be measured but also the exchange of K\textsuperscript{1} in addition to \(J_{\text{Na,K pump}}\) or \(J_{\text{Ksc}}\). Therefore, radioisotopes are often used in conjunction with specific blockers, for

**Table 4. Flux equations**

<table>
<thead>
<tr>
<th>Compartment</th>
<th>Potassium</th>
<th>Fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell (c)</td>
<td>(\frac{dK^1_c}{dt} = J_{\text{Na,K pump}} + J_{\text{Ksc}})</td>
<td>(\frac{dV_c}{dt} = J_{\text{ppc}})</td>
</tr>
<tr>
<td>Interstitium (s)</td>
<td>(\frac{dK^1_s}{dt} = J_{\text{Kps}} - J_{\text{Kc}})</td>
<td>(\frac{dV_s}{dt} = J_{\text{pp}} - J_{\text{Fp, out}} - J_{\text{Fp}})</td>
</tr>
<tr>
<td>Capillary plasma (p)</td>
<td>(\frac{dK^1_p}{dt} = J_{\text{K, in}} - J_{\text{K, out}} - J_{\text{Kps}})</td>
<td>(\frac{dV_p}{dt} = J_{\text{pp, in}} - J_{\text{Fp, out}} - J_{\text{Fp}})</td>
</tr>
</tbody>
</table>

See Tables 2 and 3 for definitions.
instance, digitalis which blocks the Na\(^+\)-K\(^+\) pump, various channel blockers, or blockers of other transport proteins (e.g., bumetanide, amiloride).

Another approach was taken by Sjøgaard et al. (598) and more recently by Lindinger and Hawke (410). They perfused the isolated rat hindlimb with blood containing \(^{42}\)K\(^+\) at rest and during continuous stimulus at 4 Hz. \(^{42}\)K\(^+\) was equilibrated for 30 min with the extracellular space. The specific activity of \(^{42}\)K\(^+\) in the intracellular space was expected to remain negligible throughout the experiments, and therefore, no \(^{42}\)K\(^+\) release from the intracellular space would bias the data (646). With the assumption of a constant extracellular \(^{42}\)K\(^+\) content, \(J_{Na-K\hspace{0.1cm}pump}\) was calculated as the product \([^{42}\text{K}^+]_{v-a}\cdot[K^+]_{a}\cdot[^{42}\text{K}^+]_{a}\cdot J_{Fa\hspace{0.1cm}in}\), where \([K^+]_{a}\cdot[^{42}\text{K}^+]_{a}\) is the inverse of the specific activity of \(^{42}\)K\(^+\). The intracellular K\(^+\) dissipation rate was calculated as \([K^+]_{v-a}\cdot J_{Fa\hspace{0.1cm}in}\), assuming that little or no accumulation of \([K^+]_{a}\) occurred, which implies that \(d[K^+]_{a}\cdot dr^{-1}\) equals K\(^+\) loss rate. The assumption that a possible variation in intracellular K\(^+\) and \(^{42}\)K\(^+\) does not influence the results significantly was justified by the almost unrestricted diffusion across the capillary wall, which does not allow for large gradients between \([K^+]_{a}\) and \([K^+]_{v}\) in the well-perfused muscle, and the small size of the interstitial space, i.e., \(\sim 10\%\) of total muscle water content as mentioned above. From these two estimates, \(J_{Ksc}\) was calculated.

An alternative is to monitor continuously \([K^+]_{v}\), after a sudden change in \(J_{Ksc}\) or \(J_{Na-K\hspace{0.1cm}pump}\). A sudden stepwise change in \(J_{Ksc}\) occurs at the onset and cessation of exercise and sudden changes in cardiac beating frequency. The onset of exercise is accompanied by a sudden appearance of AP. In skeletal muscle, this estimate will in addition require knowledge of the fiber recruitment and firing frequency.

By a similar method, beat-related \(J_{Ksc}\) was estimated in pig hearts on the basis of recordings of \([K^+]_{v}\) in the coronary sinus during sudden increments and decrements of beating frequency (177). Also, \(^{42}\)K\(^+\)-K\(^+\) pump rate has been estimated in the pig heart after abrupt injection of ouabain into the coronary artery to suddenly block the Na\(^+\)-K\(^+\) pump (176). As is the case with all these methods, estimates of \(J_{Ksc}\) cannot always be related to the actual size of the repolarizing K\(^+\) currents associated with an AP. In skeletal muscle, this estimate will in addition require knowledge of the fiber recruitment and firing frequency.

With maintained stimulation of the muscle, \(J_{Na-K\hspace{0.1cm}pump}\) will increase, and in the heart in some circumstances in skeletal muscle, eventually \(\Delta J_{Ksc} = -\Delta J_{Na-K\hspace{0.1cm}pump}\) (disregarding a possible transport component by other transporters). If the firing frequency is then suddenly reduced (exercise is stopped or heart rate reduced), a similar line of reasoning will lead to the conclusion that \(\Delta J_{Na-K\hspace{0.1cm}pump} = -d[K^+]_{v}\cdot dr^{-1}\). In other words, therefore, initial changes in \([K^+]_{a}\) may under certain circumstances be used to estimate both the increase in K\(^+\) release associated with increased firing frequency and the accompanying rise in K\(^+\) uptake rate provided by the Na\(^+\)-K\(^+\) pump.

The rate of K\(^+\) loss or gain for the whole muscle can be calculated if fluid volumes in the muscle remain fairly constant for a short period (410). Also in many circumstances \([K^+]_{a} \sim [K^+]_{p} = [K^+]_{v}\). Normally, lymph flow is very low compared with plasma flow and may often be ignored. In this case, and assuming constant muscle water content, i.e., \(J_{Fa\hspace{0.1cm}in} - J_{Fv\hspace{0.1cm}out} = 0\), total K\(^+\) output will equal \(J_{Fa\hspace{0.1cm}in}\cdot [K^+]_{v}\), and rate of K\(^+\) loss will be equal to \(J_{Fa\hspace{0.1cm}in}\cdot [K^+]_{v} - [K^+]_{a}\) or \(J_{Fa\hspace{0.1cm}in}\cdot [K^+]_{v-a}\) (arterial plasma flow times the venoarterial concentration difference for K\(^+\)). If, in addition \(d[K^+]_{v}\cdot dr^{-1}\) is \(dK^+_v\cdot dr^{-1} = 0\), (no accumulation of K\(^+\) in the interstitial space or in the vascular volume) so that \(-J_{Ksc} = J_{Na-K\hspace{0.1cm}pump} + J_{Ksc} + J_{Kl\hspace{0.1cm}out} = J_{Ka\hspace{0.1cm}in} - J_{Kv\hspace{0.1cm}out} - J_{Kos}\), loss rate will reflect the balance between uptake and release rates across the sarcolemma since the equation transforms to \(J_{Ksc} + J_{Na-K\hspace{0.1cm}pump} = J_{Ka\hspace{0.1cm}in} - J_{Kv\hspace{0.1cm}out} - J_{Kl\hspace{0.1cm}out} \sim J_{Fa\hspace{0.1cm}in}\cdot [K^+]_{v-a}\). Because \(J_{Na-K\hspace{0.1cm}pump} + J_{Ksc} = dK^+_v\cdot dr^{-1}\) intracellular K\(^+\) dissipation rate equals arterial plasma flow times \([K^+]_{v-a}\) provided muscle volumes and extracellular K\(^+\) content inside the muscle remain
unchanged. Naturally with vascular occlusion or occluded microcirculation during contraction, there is no flow and whole muscle K+ gain or loss rates will be zero.

The existence of an interstitial space has important implications for the study of K+ fluxes in muscle in that this space serves as a reservoir for K+ storage, in electrical terms a capacitance. There are two important consequences of this that we would like to highlight. First, loss or gain of K+ for the whole muscle will not reflect intracellular dissipation or accumulation of K+ if interstitial K+ is changing. Second, [K+]i will only to a small extent vary with flow through the muscle.

Previous authors have indirectly inferred that cellular uptake and release of K+ can be deduced from measurements of [K+]v and flow (332, 462). As pointed out by Hallén and Sejersted (254), this can only be done if [K+]i is constant and in equilibrium with [K+]v. If [K+]i is not stable, it means that K+_v is constantly changing. Thus, at the onset of exercise, all the K+ leaving the muscle cells will initially accumulate in the interstitial space, but soon K+ will also leave the muscle by the perfusing blood. Thus initial [K+]v-a will grossly underestimate the rate of release of K+ from the muscle cells. The same holds true at cessation of exercise when [K+]v-a will grossly underestimate K+ uptake since [K+]v is falling rapidly. At these points the release/uptake rate for K+ will therefore actually be 

\[ \Delta \text{J}_{K_a} = d[\text{K}^+]_v \cdot dt^{-1} + d[\text{K}^+]_t \cdot dt^{-1} \]

Because V_t is small relative to V_v and [K+]v ~ [K+]t, as argued above, the relationship can be written as

\[ \Delta \text{J}_{K_a} \cdot V_v^{-1} = d[\text{K}^+]_v \cdot dt^{-1} \]

What is more, small variations in [K+]v when exercise is continued for several minutes can contribute considerably to the calculated loss (252).

Interestingly, because the intracapillary volume is roughly 10 times smaller than the interstitial volume both in skeletal muscle and heart muscle, the magnitude of the flow, \( J_{FA, in} \), will not greatly affect the initial rate of rise or fall of [K+]v at onset and cessation of exercise (254). This is due to the fact that the rate at which K+ is removed by the flow is small compared with the rate at which K+ rises or falls initially. To avoid the confounding effect of flow on the rate of rise or fall of [K+]v, initial rates were estimated in several studies by means of K+-selective electrodes in the femoral vein of exercising human subjects (246, 252–254, 568). Also, in these experiments [K+]v rose or fell linearly over 5–10 s (see Fig. 1), indicating that even a rapid rise of muscle blood flow as seen at the onset of exercise or a maintained high muscle blood flow at the end of exercise do not seem to affect the rate of change of [K+]v, to any great extent even over a time period of just a few seconds (254). At the other hypothetical extreme, the rate of rise of [K+]v would vary in proportion to flow if the interstitial volume was very small, which is clearly not the case.

The various ways of estimating K+ translocations as outlined above, and the corresponding prescribed assumptions, make it clear that there is no simple relationship between rate of K+ loss from the whole muscle and rates of K+ release and uptake at the sarcolemma. Furthermore, estimates of [K+]v-a and blood flow, \( J_{FA, in} \), can only be used to deduce reliable data on uptake and release at the cellular level when K+_v is constant. More reliable estimates of \( J_{K_a} \) and \( J_{Na-K\text{ pump}} \) are provided by the use of isotopes or by estimates of initial rates of concentration changes following sudden changes in electrical activity. However, there is certainly a need for better ways of measuring [K+]s directly.

D. Maintenance of Resting Plasma K+ Concentration

The resting plasma [K+] in the general circulation ([K+]v_mix or [K+]p_mix or [K+]t) is kept within narrow limits (3.5–5.5 mM) (191, 622). Interestingly, some investigators claim that [K+]s in resting skeletal muscle is slightly higher than [K+], (404, 636) (G. L. Nilsen and O. M. Sejersted, personal communication). The reason for this is unclear, since the interstitial Cl− concentration is not correspondingly low as one would expect for a Donnan effect of negatively charged macromolecules in the interstitium. The kidneys are responsible for the long-term clearance from plasma of ingested or injected K+, and they also respond with increased fractional reabsorption of K+ when intake is low. Extrarenal tissues like muscle which contains the largest exchangeable pool of K+ probably play the most important role in the acute buffering of a K+ load and control of [K+]s. Clearly, exchange kinetics for K+ between the extracellular and intracellular spaces differ greatly depending on whether, for example, the muscle is active, stimulated by hormones, or well perfused. These and other examples are discussed in subsequent sections of this review. Here we briefly go through the data relating to the resting condition.

Several hormones cause a decrease of [K+]v_mix. This effect is well established for epinephrine, \( \beta \)-agonists, and insulin (137, 139, 284, 639, 656), although D’Silva (158a) originally reported an increase of [K+]mix with epinephrine. These effects have already been reviewed on a number of occasions (see for instance Refs. 48, 135).

At rest, K+ is taken up quite slowly by the extrarenal tissues. After about 1-h infusion of KCl to normal subjects, only 15% had been excreted by the kidneys whereas 35% was still located in the extracellular space (540), which contrasts the normal extracellular fraction of just 2%. Lindinger et al. (409) showed that 3.5 h after ingestion of KHCO₃ only 37% had been taken up by peripheral tissues. The mechanism for slow cellular uptake remains, however, unclear. Adrenalectomy reduces the extrarenal disposition rate (138, 586), and part of the disposition rate is recovered by administration of aldosterone (49). In addi-
tion, β-adrenoceptor blockade reduces the rate of extrarenal uptake of infused K⁺, and simultaneous infusion of β-agonists may completely ameliorate the rise of plasma [K⁺], (136). Kubota and Inghar (375) showed that β₂-adrenoceptor stimulation increased extrarenal disposal rate of K⁺, and disposition rate was higher in hyperthyroid animals, but there was no interaction between the two hormones. Stimulation of α-adrenoceptors has the same effect as β-adrenoceptor blockade (703). Finally, absence of insulin leads to decreased tolerance to infused K⁺ (138, 139). Possibly, both insulin and catecholamines are instrumental in facilitating the extrarenal tissues to temporarily store ingested K⁺ until it is excreted by the kidneys.

Increased uptake of K⁺ in resting muscle may be achieved by increased Na⁺-K⁺ pump rate or decreased K⁺ release from the cells. As discussed below, Na⁺-K⁺ pump rate can be increased slightly by a rise above normal of [K⁺]ₑ. Also, conductance of the inwardly rectifying K⁺ channel is increased by higher extracellular [K⁺]. These two effects can possibly explain muscle uptake of K⁺ when extracellular [K⁺] rises. However, in isolated cardiac Purkinje fibers, [K⁺]ₑ did not change when the concentration in the bath was varied, thus indicating that stimulation of the Na⁺-K⁺ pump did not occur under these in vitro conditions. (475). What these findings indicate is that a basal level of several hormones, including epinephrine, insulin, and aldosterone, is in fact required for the muscle cells to respond with a K⁺ uptake when the [K⁺]ₑ is raised.

III. TRANSPORT ACROSS THE SARCOLEMMA

In relation to exercise, the short-term regulation of K⁺ is essential. This section looks at processes that are vital for rapid K⁺ shifts across the cell membrane. Membrane is here used as a general expression for the cell membrane, as in E_mem, membrane transport proteins, etc. The cell membrane of muscle cells, by definition, comprises the sarcolemma as well as the much larger t-tubular membrane. The specific properties of the t-tubular structure are dealt with in section IV D. Many of the same membrane proteins are present in the t tubules and the sarcolemma.

A. K⁺ Channels in Muscle

1. K⁺ channel superfamilies

There is an extremely large diversity of K⁺ channels in striated muscle, especially in the heart, and several reviews have recently appeared (36, 65, 87, 131, 133, 319, 487a, 489–491, 625, 627, 641, 698). There seem to be two dominating superfamilies of channel-forming proteins that have been named Kv (v for voltage sensitive) and Kir (ir for inward rectifier). In addition, a third type of protein, the so-called minimal or minK, has been identified as an integral part of some K⁺ channels. Finally, Kv channels have associated β-subunits that speed up inactivation (531). To add to the complexity, a novel cardiac two-pore background K⁺ channel was recently cloned (347).

In the voltage-sensitive Kv superfamily, each peptide chain has the classical structure of six membrane-spanning segments which is also typical for Na⁺ and Ca²⁺ channels (92, 133). Four peptide chains associate to form voltage-sensitive channels (homo- or heterotetramers) (444). Four subfamilies have been identified within this superfamily (Shaker, Shab, Shaw, and Shal), and there are several varieties within each subfamily. Only peptide chains from the same subfamily can form functional channels, with the possible exception of association with minK (see below).

In the Kir superfamily, each subunit is much smaller, having only two membrane-spanning regions and lacking the positively charged segment that provides voltage sensitivity (157). These proteins can also form homo- or heterotetramers with a central channel that typically exhibits inward rectification (36, 374). There are at least six subfamilies (490).

The minK (or Iₖ) was recently cloned and has only one transmembrane segment (36). The minK protein alone cannot associate to form channels (487a). They can associate with Kv proteins to form a delayed rectifier belonging to the voltage-sensitive channels (32, 456, 556). Interestingly, the minK gene seems to be expressed preferentially in the conducting system of the mouse heart (379).

The variety of K⁺ channels is further extended by alternative splicing, something that seems to be quite important in at least some species (319, 444, 710). Finally, the variability arises from the fact mentioned above that various subunits with different properties can associate to form a functional channel (133, 444). In this way, for instance, ligand-controlled gating can be introduced in voltage-sensitive channels (374). This is in contrast to Na⁺ and Ca²⁺ channels where the corresponding four peptide chains are actually linked so that the channel-forming protein is one large as opposed to four different smaller proteins. A brief account of the two most important classes of channels will be given below, since it is the properties of these channels that determine the K⁺ release from the heart and from active skeletal muscle.

2. Voltage-sensitive channels

These channels show voltage- and time-dependent activation but only some show voltage-dependent inactivation. The great diversity of voltage-sensitive K⁺ channels is reflected in the different voltage sensitivities and
kinetics. In both heart and skeletal muscle, the delayed rectifier plays a role for the repolarizing K+ current ($I_{K}$) during action potentials, and this in turn contributes to K+ release during muscle activity. This channel is quite abundant in skeletal muscle and, moreover, it displays significantly more rapid kinetics than in the heart, which in one way accounts for the much shorter AP (298, 579, 625). At $E_{m}$ values positive to −70 mV, the delayed rectifier in mouse extensor digitorum longus muscles (EDL) showed inactivation that was half-maximal at −50 mV (298). In the heart, one finds both a rapid current that inactivates ($I_{K,r}$) and a slow current that does not show inactivation ($I_{K,s}$) (557). There is also a rapidly activating, nonactivating K+ current ($I_{Kur}$; Ref. 487a). However, there are species and/or regions of the heart where the expression of these channels is very low (36, 558). In skeletal muscle, there seems to be variable expression of some channel genes depending on fiber type and pattern of excitation (662). To some extent, these channels show the classic behavior described by Hodgkin and Huxley (301). They slowly activate when the cells become depolarized and exhibit some outward rectification, which means that they easily pass an outward current. It has been shown that these channels are subject to hormonal control in the heart, but it is presently unclear whether this is the case in skeletal muscle. The channel proteins can be phosphorylated probably both by protein kinase A and protein kinase C, which both increase the current (313, 640, 665). The Ca$^{2+}$-activated K+ channels [$I_{K(Ca)}$] are further examples of channels common to both heart and skeletal muscle. They are activated by both intracellular Ca$^{2+}$ ($[Ca^{2+}]_i$) and depolarization, but closed by low intracellular pH (40, 83, 392, 464, 465, 516). Barrett et al. (35) found a strong interaction between Ca$^{2+}$ and voltage so that at −50 mV openings were recorded at 1 μM Ca$^{2+}$, whereas at +50 mV only 0.01 μM Ca$^{2+}$ was required. There are two types of Ca$^{2+}$-activated K+ channels, one of which has very large conductance (670). A detailed analysis of the gating kinetics of the large-conductance Ca$^{2+}$-activated K+ (BK) channels from rat skeletal muscle was recently published (541). These channels may be quite important for the repolarization since [Ca$^{2+}$]$_\text{c}$ is raised during contraction, although their voltage dependence is much less than that of the delayed rectifiers. These channels can open at lower [Ca$^{2+}$]$_\text{c}$ concentrations than previously expected, although only during long-lasting depolarizations, and hence they can be of importance either during sustained muscle activity or in exhausted fibers (317). It was recently proposed that in the heart, the L-type Ca$^{2+}$ current could activate these K+ channels either directly or indirectly via the triggering of Ca$^{2+}$ release from the sarcoplasmic reticulum (666). In line with the properties of these channels, Fink and Lüttgau (198) described a large increase in membrane K+ conductance in metabolically exhausted frog skeletal muscle fibers and later ascribed this to a rise of [Ca$^{2+}$]$\text{c}$ (197, 435). However, it is possible that part of the conductance increase these authors observed also could be ascribed to opening of ATP-dependent K+ channels (see sect. II A 3).

In heart muscle, a Na$^+$- and voltage-sensitive K+ current has also been identified [$I_{K(Na)}$] (342, 433). This channel has yet to be cloned. Its role in normal situations is unclear, since [Na$^+$]$_\text{c}$ in the range of 20 mM is required for opening (653), and it was recently identified in guinea pig, but not in rat ventricular myocytes (393).

Finally, an important class of channels present in the heart gives rise to the transient outward current ($I_{to}$) (119, 711). They rapidly activate and inactivate on depolarization and contribute to the early “notch” in the cardiac AP. In actual fact, in the regions of the heart where they are present (that is especially in the epicardium, Ref. 36), these channels seem to be quite important for the level of the plateau phase in heart cells, thereby determining in part both the size of the Ca$^{2+}$ current and the length of the AP (625).

3. Inward rectifiers

Inward rectification means that these channels have a high conductance for inward K+ currents and that K+ currents in the outward direction are slower. As described in section IV, it is only when cells are hyperpolarized by means of the Na$^+$-K+ pump to potentials more negative than the K+ equilibrium potential ($E_{K}$) that the K+ current is inward. Therefore, in resting cells, the normal direction of the K+ current is in fact outward. The voltage-sensitive gating mechanism of these channels is very different from the voltage sensitivity described above.

The inward rectifiers (belonging to the Kir family) comprise three channel types or currents. The classic inward rectifier (IRK) gives rise to the $I_{K1}$ current. The ATP-sensitive K+ channel opens [$I_{K(ATP)}$ current] when intracellular ATP concentration ([ATP]$_c$) is lowered (501), while the acetylcholine-sensitive K+ channel [muscarinic, $I_{K(ACh)}$ current] opens through a direct interaction with G proteins (380, 431). The latter is found only in the heart, whereas the two others are highly expressed in tissue both in heart and skeletal muscle (315, 316, 446, 501, 615). The inward rectifiers do not possess the positively charged S4 transmembrane segment as do the voltage-sensitive channels. Instead, block of the channel is achieved by intracellular Mg$^{2+}$ or polyamines, such as spermine, spermidine, or putrescine (490). The rectification is rapid, and there is no inactivation, which means that the channels remain open or closed as long as voltage, ion concentrations, and ligand control remain the same. Apparently, in the heart, control of this channel can also be achieved through its anchoring to the cytoskele-
ton and through a slight β-adrenergic inhibitory effect (370, 371, 449).

The most important effect of these channels is to stabilize the $E_{m}$, which means that when they are open more current will be needed to alter $E_{m}$ since membrane resistance is low. As described in section IV A, opening of these channels will force the $E_{m}$ to become very close to $E_{K}$ since they are highly selective for $K^+$. The strong rectification of $I_{K1}$ means that it passes little current throughout the AP. However, some outward current can be passed as repolarization approaches the resting $E_{m}$ and in this way this channel also contributes to the exercise-induced $K^+$ release (see sect. IV C). It is first of all responsible for most of the basal $K^+$ conductance (272, 579). The two other channels exhibit much weaker rectification, which means that ligand-gated control predominates and that outward current is also passed (502). The effect of opening these ligand-gated channels in resting cells may therefore only be a very small transient $K^+$ release until the $E_{m}$ is stabilized at a more negative level. However, because more current will have to be passed to depolarize cells sufficiently to trigger an AP, cell excitability is reduced, and cells may become unexcitable (196, 559). Furthermore, because they conduct outward currents, the inward $Na^+$ current (and $Ca^{2+}$ current in the heart) will be short circuited during the AP, and more inward current will have to be passed to elicit an AP. Hence, opening of these channels may well cause enhanced $K^+$ release during muscle activity, something which was clearly shown during β-adrenergic stimulation of the heart (16, 177).

In recent years there has been much focus on the ATP-sensitive $K^+$ channels. They open at [ATP]$_{c}$ well below the millimolar range (395, 501), although openings are seen at 2 mM (615). Such low [ATP]$_{c}$ are not seen under physiological conditions nor in the ischemic myocardium (12). Several additional factors are important. First, the $K_{ATP}$ channels are pH sensitive (although less so in heart compared with skeletal muscle) so that when intracellular pH is lowered open probability is increased (130, 132, 395). Second, it has been pointed out that the channels are so numerous that even a small increase in the probability of opening that occurs with quite small reductions of the [ATP]$_{c}$ may be sufficient to cause a large current (488, 685). It has also been argued that the [ATP] in the subsarcolemmal space may be different from that in the cytosol (304, 682). However, recently, in an ingenious experiment on heart cells where the $Na^+\cdot$K$^+$ pump was manipulated to run in both normal and reverse mode to vary the subsarcolemmal [ATP], Priebe et al. (521) compared results of cell-attached and isolated patch-clamp experiments. They concluded that variable consumption of ATP (and in the reverse mode production of ATP by the pump) will transiently affect $I_{K_{ATP}}$, but the effect was passing and they therefore concluded that the submembrane [ATP] is readily controlled by the cytosolic ATP pool. In a recent study, Kabakov (336) also noted a tight relationship between $Na^+\cdot$K$^+$ pump rate and $I_{K_{ATP}}$, and that a stable submembrane depletion of ATP seemed to occur that could explain the activation of the ATP-sensitive $K^+$ channels. Interestingly, this author also points out that the interaction of these two membrane proteins via the submembrane [ATP] will affect the way the $Na^+\cdot$K$^+$ pump contributes to $E_{m}$. In accordance with these results, it was recently confirmed that inhibition of the $Na^+\cdot$K$^+$ pump with digitalis in metabolically stressed cardiomyocytes will also close the ATP-sensitive $K^+$ channels (644). Third, it has recently been shown that these channels may also open during mechanical stress (648), possibly through their anchorage to the cytoskeleton (216). Fourth, at least in heart, although not necessarily in skeletal muscle, MgADP is an important regulator of channel activity, since ADP can antagonize channel closure by ATP (131). MgADP binds to the sulfonylurea receptor that is associated with the channel protein. Thus the $K_{ATP}$ channel is highly sensitive to the [ATP]/[ADP], which has caused some investigators to call it “metabolic sensor” (641). All these mechanisms may help explain why $K_{ATP}$ channels are responsible for the great increase of membrane $K^+$ conductance seen during ischemia and in metabolically exhausted skeletal muscle fibers (212, 228, 366, 435, 685).

B. $Na^+\cdot$K$^+$ Pump in Muscle

The $Na^+\cdot$K$^+$ pump is instrumental in maintaining low [Na$^+$]$_{c}$ and high [K$^+$]$_{c}$ and hence muscle function. The first point to be made is that the amount of active pump proteins will determine the overall pumping capacity of the tissue. This is subject to long-term regulation as discussed in section v, but here we discuss this pump capacity is large enough in muscle tissue to cope with the $K^+$ release through $K^+$ channels. Second, short-term control of pump rate is achieved through 1) its sensitivity to [K$^+$]$_{c}$ and [Na$^+$]$_{c}$, 2) the voltage dependence, 3) hormonal control, 4) its dependence on an ATP supply, and 5) possibly through some hitherto unrecognized control mechanisms.

1. Tissue content of $Na^+\cdot$K$^+$ pumps and ion-pumping capacity

The issue of tissue content of $Na^+\cdot$K$^+$ pumps has been extensively reviewed (99, 103, 560), so let it here suffice to mention that the number of [3H]ouabain binding sites that reflect the $Na^+\cdot$K$^+$ pump density in skeletal muscle is in the range of 300 pmol/g wet tissue wt and slightly higher in fast-twitch muscles compared with slow-twitch muscles (see sect. v). In the heart, the tissue content is much higher, approaching 2,000 pmol/g in rats.
and 200–600 nmol per second per pump protein, these levels translate into maximum pump capacities for K\(^+\) of \(~100\) and 200–600 nmol \(\cdot\) g\(^{-1}\) \(\cdot\) s\(^{-1}\) (6 and 12–36 mmol \(\cdot\) kg\(^{-1}\) \(\cdot\) min\(^{-1}\)) in skeletal muscle and heart, respectively. Several studies have confirmed that these pump rates calculated from \(^{3}H\)ouabain binding measurements can actually be measured in the intact tissue after Na\(^+\) loading of the cells to [Na\(^+\)]\(_{c}\) levels that saturate the internal site of the pump protein (105, 574). From these data it is clear that skeletal muscle has a much lower reserve capacity of the Na\(^+\)-K\(^+\) pump as compared with the heart, since stimulation frequencies are often in the range of 10–30 Hz, although they can even exceed 100 Hz in fast muscles, at least for short periods (41, 242, 341, 609). In contrast, stimulation frequencies in the heart are at maximum between 3 and 10 Hz depending on species. Therefore, as discussed in more detail in section VI B, Clausen (100) has calculated that under certain conditions of sustained high-frequency stimulation of skeletal muscle K\(^+\) release may actually exceed pump capacity. This may cause considerable dissipation of K\(^+\).

It has been generally held that muscle activity does not acutely change the number of active Na\(^+\)-K\(^+\) pumps in the muscle. However, recently, Juel et al. (333a) reported a significant translocation of Na\(^+\)-K\(^+\) pumps to the plasma membrane during exercise. Provided this is correct, it is an important way of increasing the maximum pump capacity. In the following we focus on other means of increasing Na\(^+\)-K\(^+\) pump rate.

\section{2. Na\(^+\)-K\(^+\) pump control mechanisms}

\subsection{A) The concept of Na\(^+\) pump lag} An important issue is whether the Na\(^+\)-K\(^+\) pump is activated quickly enough to cope with increases in K\(^+\) release in situations where the maximum pump capacity is not taxed. In excitable tissues this is clearly not the case. In nervous tissue as well as in heart and skeletal muscle, the sudden increase of outward repolarizing K\(^+\) currents associated with higher firing frequency clearly exceeds pump-mediated K\(^+\) uptake rate for a period (195, 385, 626). Eventually, pump-mediated uptake may or may not match release. During the transition from one steady state to another with a higher firing frequency, there is a transient dissipation of intracellular K\(^+\) (177, 252, 314, 332). To explain this slow activation of the pump, the concept of the Na\(^+\) pump lag was put forward (384, 706). It was based on the assumption that activation of the pump was merely due to the rise in [Na\(^+\)]\(_{c}\) that followed electrical activity (706). Also, the rapid extracellular accumulation of K\(^+\) has been implied to activate the pump, and also other means of activating the pump, such as electrical and mechanical activity, have been proposed, (184, 186, 496).

\subsection{B) Na\(^+\)-K\(^+\) pump stimulation by K\(^+\) and Na\(^+\)} Clearly, very important control of Na\(^+\)-K\(^+\) pump rate is achieved by its sensitivity to [K\(^+\)]\(_{s}\) and [Na\(^+\)]\(_{s}\). This issue was recently reviewed by Semb and Sejersted (576). The stimulation of the pump by [K\(^+\)]\(_{s}\) seems to have a \(k_{0.5}\) (i.e., the [K\(^+\)]\(_{s}\) that stimulates the Na\(^+\)-K\(^+\) pump to 50\% of its maximum pump rate) of 0.8–1.5 mM. This value is obtained in isolated cells where the problem of extracellular clefts and diffusion through restricted space in multicellular preparations have been eliminated (117, 486, 527). Hence, at a normal [K\(^+\)]\(_{s}\) of 4 mM, the extracellular K\(^+\) site of the enzyme will be \(~80\%) saturated. In this way, pump activation due to extracellular K\(^+\) accumulation cannot amount to more than 20\%. On the other hand, a reduction in [K\(^+\)]\(_{s}\) can cause a significant reduction in Na\(^+\)-K\(^+\) pump rate. This may be relevant during recovery after exercise, when [K\(^+\)]\(_{s}\) below normal resting value is observed. In fact, the sensitivity of the Na\(^+\)-K\(^+\) pump to [K\(^+\)]\(_{s}\) may in this situation prevent the pump from lowering [K\(^+\)]\(_{s}\) too much. In a clinical setting, during hypokalemia due to renal or gastrointestinal losses, reduced pump activity can explain several of the symptoms.

Pump sensitivity to [Na\(^+\)]\(_{s}\) is somewhat less clear. There are at least three reasons why data based on experiments with the isolated enzyme may not be extrapolated to the intact tissue. First, vectorial transport with different concentrations on each side of the membrane cannot be replicated in the vial. Second, there might be important limitations to diffusion in the subsarcolemmal space of intact cells (fuzzy space) (576), which is likely to disappear when the membrane is ruptured. Third, the Na\(^+\)-K\(^+\) pump is probably anchored to the cytoskeleton by ankyrin (144, 487). This connection may be important for function (382) and will probably be ruptured in isolated membrane fractions as well as in excised giant patches. As reviewed by Semb and Sejersted (576), investigations on intact heart cells from several species indicate that when expressed in terms of the Hill equation constants, \(k_{0.5}\) seems to be \(~15\) mM in terms of concentration, and the Hill coefficient is \(~2\). However, in the rat heart, \(k_{0.5}\) is probably higher, which tallies with the higher resting [Na\(^+\)]\(_{s}\) in heart cells from this species (575). At the time of publishing, we are only aware of one preliminary study in which [Na\(^+\)]\(_{s}\) was measured directly in the rat soleus muscle, and \(k_{0.5}\) was found to be slightly higher than 15 mM (57).

One important consideration arises from this estimate, namely, how in resting skeletal muscle is it possible that Na\(^+\)-K\(^+\) pump rate is as small as 4–5\% of its maximum capacity (94, 99, 105, 188). With ion-selective micro-electrodes, [Na\(^+\)], in resting mouse soleus muscle has been reported at 11.1 and 12.7 mM (329, 331), and at about the same level in frog muscle (26). Doing repeated im-
palemments (n = 60) on isolated rat soleus muscles at 37°C with a conventional and an Na⁺-selective electrode. Bjørklund and Sejersted found $E_m$ to be −67.2 mV and $[\text{Na}^+]_c$ 18.5 mM (unpublished data). With more indirect techniques the estimates of $[\text{Na}^+]_c$ are between 20 and 30 mM in rat muscles (183, 496) and in human muscle between 6 and 13 mM (595) and −21 mM (588). Therefore, it would be safe to say that $[\text{Na}^+]_c$ is probably in the range of 15 mM in resting slow-twitch skeletal muscle. In fast-twitch muscle, $[\text{Na}^+]_c$ is significantly lower (183). With a $k_{0.5}$ of 15 mM, Na⁺-K⁺ pump rates should be ~50% and not 4–5% in the soleus muscle. Unless a large fraction of the pumps are silent, it follows that the $k_{0.5}$ for pump stimulation by $[\text{Na}^+]_c$ must be considerably higher than 15 mM in resting skeletal muscle. So far, this is in seeming contrast to existing estimates. In contrast, in quiescent heart tissue, $[\text{Na}^+]_c$ is well below 10 mM (143, 396, 574, 580), which fits with a $k_{0.5}$ of 15 mM and a basal Na⁺-K⁺ pump rate in the order of 10% of maximum (575). In skeletal muscle, there are several observations that fit with a $k_{0.5}$ of ~15 mM when the muscle is active. For instance, maximum pump rates seem to be achieved with $[\text{Na}^+]_c$, not much higher than 30 mM (496, 574). Following this simple line of reasoning, it would make sense to assume that the sensitivity of the Na⁺-K⁺ pump for Na⁺ is probably regulated in skeletal muscle. Further evidence for this is presented below.

**C) FACTORS THAT CAN AFFECT LOCAL CONCENTRATIONS OF K⁺ AND Na⁺ IN THE CELL.** Let us first examine the basic concept of the Na⁺ pump lag with a constant $k_{0.5}$. The rate of activation that can be achieved by an increase of $[\text{Na}^+]_c$, is dependent on the ratio between the increase in K⁺ release (release per AP multiplied by the increase in firing frequency) and the amount of Na⁺-K⁺ pumps available in addition to the Na⁺ sensitivity of the pump. In Figure 4 we present some simulation data (571). In Figure 4, A and C, Na⁺-K⁺ pump activation has been plotted as a function of time after an increase in heart rate of 3 Hz and an increase in muscle firing frequency of 30 Hz, provided increased $[\text{Na}^+]_c$ was the only pump stimulus. In both tissues the starting $[\text{Na}^+]_c$ was set at a level equal to 8 mM. This value is probably slightly higher than in resting heart cells in which $[\text{Na}^+]_c$ is probably in the range of 15 mM (574). In contrast, in quiescent heart tissue, $[\text{Na}^+]_c$ is well below 10 mM (143, 396, 574, 580), which fits with a $k_{0.5}$ of 15 mM and a basal Na⁺-K⁺ pump rate in the order of 10% of maximum (575). In skeletal muscle, there are several observations that fit with a $k_{0.5}$ of ~15 mM when the muscle is active. For instance, maximum pump rates seem to be achieved with $[\text{Na}^+]_c$, not much higher than 30 mM (496, 574). Following this simple line of reasoning, it would make sense to assume that the sensitivity of the Na⁺-K⁺ pump for Na⁺ is probably regulated in skeletal muscle. Further evidence for this is presented below.

![Simulation of Na⁺-K⁺ pump activation in heart and skeletal muscle.](http://physrev.physiology.org/)
most species, while at the same time somewhat lower than the value discussed above for skeletal muscle. These deviations do not affect the results of the simulation with regard to the main conclusions. Clearly, in heart tissue, complete adjustment of pump rate can in theory be achieved in the course of a few minutes with an increase of [Na$^+$]$_c$ of $\sim$1 mM (Fig. 4, full drawn line in A and C). In contrast to heart tissue, the number of pumps available in skeletal muscle is fewer while the firing frequency is higher. Therefore, more Na$^+$ must accumulate intracellularly to achieve adequate pump activation. However, even if the simulation allows [Na$^+$]$_c$ to rise to well above 20 mM (dotted line in C), pump activation would be slow and incomplete if this were the only stimulus for pump activation (dotted line in A). Hence, these simple simulations that utilize existing data for Na$^+$-K$^+$ pump density and K$^+$ release (incorporating firing frequency and the size of the repolarizing current) show that the original Na$^+$ pump lag concept may account for the complete compensation that occurs in heart tissue but do not provide a full explanation for Na$^+$-K$^+$ pump activation in skeletal muscle. The question therefore is whether other mechanisms contribute to pump activation during exercise.

Two factors that might affect the rise of [Na$^+$]$_c$ are cellular swelling and the existence of a subsarcolemmal fuzzy space. Several authors have reported that the water content of skeletal muscle increases with exercise as discussed in section IIIE (418, 554, 595, 600, 636), whereas in the heart there is no evidence for changes of cellular volume during increments in heart rate. The mechanism for this swelling is discussed in section IIIE. Figure 4, B and D, shows the effect of a 15% increase in cell volume (with a time constant of $\sim$14 s) and a similar cell volume reduction when the muscle activity period has ceased. Because a volume increase of the cell at start of exercise will dilute Na$^+$ and attenuate the rise of [Na$^+$]$_c$, Na$^+$-K$^+$ pump activation will be delayed as can be seen in Figure 4 comparing dotted lines in A and C with the full-drawn line in B and D. Similarly, when exercise is terminated, fluid will leave the cell and transiently cause an increased [Na$^+$]$_c$ that in theory might cause a small “overshoot” of Na$^+$-K$^+$ pump rate (Fig. 4B). All in all, the effect of swelling seems to be small; however, an important consequence of swelling is that also K$^+$ will be diluted and hence the decline of [K$^+$]$_c$ will be accelerated as K$^+$ leaves the cell. Thus, even though K$^+$ and Na$^+$ are exchanged in a ratio of 1:1 across the membrane leading to a 1:1 ratio of reciprocal changes of K$^+$ (ΔK$^+$) and Na$^+$ (ΔNa$^+$) in the cell, in terms of change of concentration the ratio will be higher and may approach 2Δ[Na$^+$]$_c$:1Δ[Na$^+$]$_c$.

Subsarcolemmal accumulation of Na$^+$ early on during periods of increased firing rates might cause a more rapid Na$^+$-K$^+$ pump activation (576). This is also illustrated in Figure 4, B and D, where the effects of swelling and a subsarcolemmal fuzzy space have been combined (dotted line). Clearly a local upconcentration of Na$^+$ in the subsarcolemmal space due to Na$^+$ coming in through the Na$^+$ channels will offset the effect of cell swelling on Na$^+$ activation of the Na$^+$-K$^+$ pump, since the intracellular Na$^+$ site of the pump will be exposed to this local and high [Na$^+$]. Thus a more rapid activation of the pump may be achieved, but with time, as a diffusion equilibrium between the subsarcolemmal fuzzy space and the rest of the cell is obtained, the effect will vanish. Again, the effect is remarkably small but may contribute to reducing loss of K$^+$ at onset of exercise by a more rapid activation of the Na$^+$-K$^+$ pump. However, in theory, an unfortunate effect will be that the Na$^+$-K$^+$ pump rate will be rapidly reduced at cessation of exercise when Na$^+$ suddenly stops coming in through the Na$^+$ channels leading to a slowing down of recovery of Na$^+$ and K$^+$. There is no evidence that Na$^+$-K$^+$ pump rate is rapidly turned off in skeletal muscle (252, 496, 658). Altogether, the effect of Na$^+$ accumulation in a fuzzy subsarcolemmal space may well contribute to more rapid pump activation, but it is of little importance after a few minutes have elapsed. Taken together, neither cell swelling by its effect on intracellular concentrations nor the existence of a fuzzy space has an important effect for the adjustment of pump rate over several minutes. By means of the simulation, a maximum of $\sim$50% of the increase in K$^+$ release could be compensated for by pump activation over a period of 5 min. These changes in [K$^+$]$_c$ and [Na$^+$]$_c$ are clearly insufficient to explain the much more rapid and complete pump activation that occurs in skeletal muscle.

D) CONTROL OF THE Na$^+$-K$^+$ PUMP RATE BY CHANGES IN PUMP AFFINITY FOR [Na$^+$]$_c$. The most pronounced effect on the pump rate was obtained by a simulated reduction in the $k_{0.5}$ for [Na$^+$]$_c$. In addition to cell swelling and subsarcolemmal Na$^+$ accumulation, the model implies a reduction of $k_{0.5}$ from 30 to 15 mM with a time constant of $\sim$15 s, and $k_{0.5}$ was simulated to rise again at cessation of exercise with a time constant somewhat above 400 s (Fig. 4, B and D). An almost identical effect was obtained by doubling the maximum pumping capacity ($V_{max}$). By carrying out these simulations, pump activity increases so that it compensated for 75% of the K$^+$ release in the course of 5 min. After 10 min, the compensation was almost complete (data not shown), which correlates well with observations on muscle K$^+$ loss in humans during bicycling (658). However, during other types of exercise, K$^+$ release never seems to be completely compensated by increase Na$^+$-K$^+$ pump rate as discussed below. Although there is no direct measurement of altered Na$^+$ sensitivity of the Na$^+$-K$^+$ pump in active as compared with resting skeletal muscle, there is strong evidence for several mechanisms both in heart and skeletal muscle that control pump rate independent of [Na$^+$]$_c$, as discussed in sections IIIB3e and IIIB3f.
E) SIGNALING MECHANISMS FOR CONTROL OF Na\(^{+}\)-K\(^{+}\) PUMP RATE. It has been shown that in the heart \(\beta\)-adrenoceptor stimulation in the presence of elevated \([\text{Ca}^{2+}]_c\), reduce the \(k_{0.5}\) of the Na\(^{+}\)-K\(^{+}\) pump, increase \(V_{\text{max}}\) and shifts the voltage dependence (see sect. \(\text{inB2r}\)) such that the pump becomes less sensitive to voltage within the physiological range (224–226). These effects were mediated by protein kinase A. More recently, the same group has reported that also \(\alpha\)-adrenergic stimulation activates the Na\(^{+}\)-K\(^{+}\) pump through protein kinase C-mediated phosphorylation (667). Isoproterenol and stimulation of the sympathetic nerves also cause a K\(^{+}\) gain of the intact heart, whereas at the same time K\(^{+}\) release and pump rates remain increased, which was interpreted as a reflection of increased pump affinity for Na\(^{+}\) (16, 175–179). Others have shown that this gain of cellular K\(^{+}\) is accompanied by a reduced \([\text{Na}^{+}]_c\) (672).

In recent papers, Dobretsov et al. (146) and Kockskämper et al. (366a) both confirmed that the cAMP-protein kinase A pathway increased Na\(^{+}\)-K\(^{+}\) pump rate in isolated rat and guinea pig cardiomyocytes, but they did not find that this effect was dependent on \([\text{Ca}^{2+}]_c\). Dobretsov et al. (146) conclude that the rate of deocclusion and release of K\(^{+}\) at the intracellular site was increased, which would appear to increase the affinity for intracellular Na\(^{+}\). Recently, Therien and Blostein (629) reported that K\(^{+}\)/Na\(^{+}\) antagonism at the intracellular site of the Na\(^{+}\)-K\(^{+}\) pump is variable from one tissue to another despite the presence of similar isoforms of the pump. They found a relationship between binding/occlusion of K\(^{+}\) by the enzyme and the apparent affinity for K\(^{+}\) as a competitive inhibitor of Na\(^{+}\) activation. They conclude that modulation of the K\(^{+}\)/Na\(^{+}\) antagonism could be a physiologically important mechanism of pump regulation especially in the heart, kidney and intestines. They did not examine the Na\(^{+}\)-K\(^{+}\) pump of skeletal muscle. We may conclude that protein kinase A-mediated phosphorylation of the Na\(^{+}\)-K\(^{+}\) pump stimulates pump rate, but that the exact molecular mechanism awaits clarification.

It was recently shown in sheep Purkinje fibers that Ca\(^{2+}\)-induced contracture caused the apparent \(k_{0.5}\) for intracellular Na\(^{+}\) to fall by 30% at the same time as \(V_{\text{max}}\) was increased (577). Furthermore, cell swelling induced by an osmotic stress will increase the apparent pump affinity to Na\(^{+}\) (682). In this case it was shown that dephosphorylation of the pump protein through a tyrosine kinase pathway caused the pump activation (46a). The same group has shown that the apparent affinity for intracellular Na\(^{+}\) in cardiac cells is also controlled by insulin, angiotensin, and aldosterone (71a, 258a, 473b). Thus, in cardiac tissue, there is good evidence for regulation of the apparent affinity for Na\(^{+}\), although this kind of regulation is probably not operative during mere increments of heart rate.

The Na\(^{+}\)-K\(^{+}\) pump in skeletal muscle is stimulated by different hormones including the catecholamines, calcitomin gene-related peptide (CGRP), insulin, amylin, and insulin-like growth factor I (20, 106, 108, 153, 376). It has been shown that increased ouabain-sensitive fluxes of Na\(^{+}\) and K\(^{+}\) can be observed after exposure of isolated muscles to various hormones in face of reduced intracellular Na\(^{+}\) (20, 106, 108, 153). However, it has become clear that these hormonal effects are insufficient in isolation to explain the rapid increase in Na\(^{+}\)-K\(^{+}\) pump rate that often occurs during exercise. The hormonal effects are too slow and, at least judging from the experiments with \(\beta\)-adrenoceptor blockade (246, 252, 645), not of sufficient magnitude to account for the increased pump rate during exercise.

As far as skeletal muscle is concerned, therefore, some central questions are posed: 1) Is there really an increase in affinity for intracellular Na\(^{+}\) during exercise? 2) By what other mechanisms could this activation occur than through hormones? There are strong arguments for control of Na\(^{+}\)-K\(^{+}\) pump affinity for Na\(^{+}\) also in skeletal muscle. First, as argued above, the low pump rate in resting muscle in spite of a high \([\text{Na}^{+}]_c\) can best be explained by a quite high \(k_{0.5}\). Second, it has recently been shown that large increases of Na\(^{+}\)-K\(^{+}\) pump rate can be achieved in the absence of a rise of \([\text{Na}^{+}]_c\) (496, 497). Third, it has recently become clear that after a period of muscle activation \([\text{Na}^{+}]_c\) is lower than the resting value for a period of many minutes (496). This lowering of \([\text{Na}^{+}]_c\) was not due to reduced back leak of Na\(^{+}\). As seen from Figure 4, provided the \(k_{0.5}\) does not return to pre-exercise value too rapidly, the simulation also implies a reduction of \([\text{Na}^{+}]_c\) below control values in the recovery period.

A similar stimulation of the Na\(^{+}\)-K\(^{+}\) pump was assumed by Medbø and Sejersted (467) when they modeled the K\(^{+}\) shifts observed during 1 min of maximum running. Here, to account for the reduction of \([\text{K}^{+}]_c\) to below normal resting value after exercise, an increase of the “proportional regulator” (i.e., the Na\(^{+}\)-K\(^{+}\) pump) was required.

There are alternative explanations for the stimulation of Na\(^{+}\)-K\(^{+}\) pump rate independent of increasing \([\text{Na}^{+}]_c\). It has been suggested that electrical activation might trigger a feed-forward pump activation that implies increased affinity for Na\(^{+}\) at the intracellular site (184, 186, 496). In these experiments it was ensured that neurotransmitter and peptide release from nerve terminals in isolated muscles did not confound the results.

It may also be worth mentioning that the Na\(^{+}\)-K\(^{+}\) pump is connected to the cytoskeleton probably via the linker protein ankyrin (487). Also, actin interacts with the Na\(^{+}\)-K\(^{+}\) pump (382) and has been found to stimulate ATPase activity in a rat kidney preparation (85). Therefore, it may be the case that the interaction with the cytoskeleton regulates Na\(^{+}\)-K\(^{+}\) pump activity.

F) VOLTAGE DEPENDENCE OF THE Na\(^{+}\)-K\(^{+}\) PUMP. Because the stoichiometry of the Na\(^{+}\)-K\(^{+}\) pump is three Na\(^{+}\}
changed for two K\textsuperscript{+}, one net charge is pumped for each cycle and not only will the pump create a current (it is rheogenic), but pump rate will also be sensitive to voltage. The rheogenic nature of the pump has been convincingly demonstrated in all tissues where it has been examined, also skeletal muscle (O. M. Sejersted, unpublished data). In a series of penetrating investigations especially from Gadsby’s group (24, 218, 219, 485, 486), the nature of the voltage dependence has been clarified. The charge moved across the membrane during a pump cycle is associated with the third Na\textsuperscript{+}, since there seems to be only two negative charges associated with the ion binding site of the protein (also see Ref. 21). Interestingly, the voltage dependence disappears when the extracellular [Na\textsuperscript{+}] ([Na\textsuperscript{+}]\textsubscript{c}) is zero, which can be explained by the channel-like outer vestibule of the pump molecule (220, 221). The effect of voltage may be important at normal [Na\textsuperscript{+}]\textsubscript{c} and within the physiological $E_m$ range. Pump rate is $\sim$10–20% below maximum at an $E_m$ of $-70$ mV. Thus, during depolarizations, pump rate may be increased, which can contribute to pump activation at onset of muscle activity. In an interesting study by Gao et al. (220), it was recently shown that in heart cells that are stimulated by isoproterenol, a rise in [Ca\textsuperscript{2+}]\textsubscript{i} will cause a marked leftward shift of the current-voltage relationship of the Na\textsuperscript{+}-K\textsuperscript{+} pump. This means that increases in [Ca\textsuperscript{2+}]\textsubscript{i} within the physiological range reduces the voltage dependence of the pump. This fits with the Na\textsuperscript{+}-K\textsuperscript{+} pump stimulation observed during Ca\textsuperscript{2+}-induced contractures in Purkinje fibers (577). Although not included in the simulation model, the release of voltage inhibition of the Na\textsuperscript{+}-K\textsuperscript{+} pump is not sufficient to explain exercise-induced pump activation in skeletal muscle. However, the hyperpolarization observed immediately after cessation of exercise (286) may significantly slow down the Na\textsuperscript{+}-K\textsuperscript{+} pump rate.

Recently, Barnashenko et al. (34) showed that also the apparent affinity for Na\textsuperscript{+} at the intracellular site of the Na\textsuperscript{+}-K\textsuperscript{+} pump is voltage dependent. During depolarization of guinea pig ventricular myocytes, the affinity for [Na\textsuperscript{+}]\textsubscript{i} increased markedly. The experiments were performed in the absence of K\textsuperscript{+} so that the competitive inhibition of the pump by [K\textsuperscript{+}]\textsubscript{i} was avoided. Even so, the data may point to an important mode of control of the Na\textsuperscript{+}-K\textsuperscript{+} pump. The same phenomenon is probably present in skeletal muscle and may be part of the feed-forward activation induced by electrical activation that is described above.

G) SUPPLY OF ENERGY TO THE Na\textsuperscript{+}-K\textsuperscript{+} PUMP. Finally, altered Na\textsuperscript{+}-K\textsuperscript{+} pump rate may occur through the supply of ATP. The $K_0.5$ for ATP is $\sim 150$ $\mu$M (213), which means that the [ATP]\textsubscript{c} needs to drop by 90% or more before appreciable inhibition of the pump will occur. This means that under normal conditions, even at the highest exercise intensities, the overall ATP supply will be sufficient for the pump. Also, during ischemia, it is questionable whether reduced ATP supply will inhibit the pump since [ATP]\textsubscript{c} never falls by more than $\sim 50\%$. At any rate, the immediate K\textsuperscript{+} release seen during anoxia and ischemia (357) cannot be ascribed to a general pump failure linked to lack of ATP, since the creatine phosphate (CrP) and ATP stores are only depleted after several minutes even in the beating heart (12). However, there are two considerations that could closer link pump rate to ATP supply. First, it has been claimed that the pump is preferentially fueled by glycolytic ATP possibly provided by membrane-bound glycolytic enzymes, as has also been claimed for the K\textsubscript{ATP} channel (234, 436, 469, 682). There is also some evidence especially from erythrocytes to suggest that this ATP represents a local pool preferentially used by the pump (304, 522). Weiss and Hiltbrand (681) used arterially perfused rabbit interventricular septa and showed that [K\textsuperscript{+}]\textsubscript{s} measured by an ion-selective microelectrode rose considerably more during conditions that inhibited glycolysis compared with conditions that inhibited oxidative metabolism, although it was only in the latter situation that contractile force was substantially attenuated. Evidence for localized membrane-associated pools of ATP has also been provided by biochemical studies of membrane fractions, indicating specific localization of glycolytic enzymes to the triads (256). Hence, to answer the question of whether the Na\textsuperscript{+}-K\textsuperscript{+} pump is indeed regulated by a subsarcolemmal or membrane pool of glycolytically formed ATP, further studies would be required.

The second possible path of ATP control of Na\textsuperscript{+}-K\textsuperscript{+} pump activity relates to the effects of pH and phosphate (P\textsubscript{i}) on the available free energy from ATP hydrolysis and the possible inhibition of the pump by P\textsubscript{i} (199, 200). Because the stoichiometry of the Na\textsuperscript{+}-K\textsuperscript{+} pump is fixed, a minimum amount of free energy from ATP is required to accomplish a complete cycle of the pump. At normal intra- and extracellular K\textsuperscript{+} and Na\textsuperscript{+} concentrations, and a $E_m$ of $-70$ mV, the efficiency of the pump is quite high. Hence, a reduction in the available free energy might cause the pump to halt even though [ATP]\textsubscript{c} is adequate (200). In line with this, a reduction of the available free energy from each ATP molecule causes pump failure (or rather pump reversal) at a less negative potential (235).

C. Na\textsuperscript{+}-K\textsuperscript{+} Pump Isoforms

The above perusal of the literature has not taken into account the fact that there are three different isoforms of both the $\alpha$- and the $\beta$-subunits of the Na\textsuperscript{+}-K\textsuperscript{+} pump protein. It is the $\alpha$-subunit that conveys the channel properties and the digitalis receptor site. The two isoforms normally present in muscle and heart are the $\alpha_1$- and the $\alpha_2$-isoforms. In rat skeletal muscle there seems to be equal amounts of the two $\alpha$-isoforms on the protein level, although the mRNA signal was much higher for the $\alpha_2$-isoforn in the soleus muscle compared with EDL (310, 633). However, such a high fraction of the $\alpha_2$-subunit in skeletal muscle does not fit with the high sensitivity of
skeletal muscle to inhibition of Na\(^+\) and K\(^+\) transport by digitalis (105), since in the rat the \(\alpha_1\)-isoform has a low affinity for ouabain. In the study by Hundal et al. (310), it was also shown that both the mRNA signal and the protein expression was almost exclusively the \(\beta_1\)-subunit in slow-twitch red muscle and \(\beta_2\)-subunit in fast-twitch white muscle. These results were largely confirmed by Thompson et al. (634), who concluded that the prevailing heterodimer configuration seems to be \(\alpha_1\beta_1\) and \(\alpha_2\beta_1\) in slow-twitch muscle and \(\alpha_2\beta_2\) in fast-twitch muscle. It is not known whether this difference has any functional consequence. The two \(\alpha\)-subunits \(\alpha_1\) and \(\alpha_2\) both have about the same affinities for extracellular K\(^+\) and intracellular Na\(^+\) (323, 482). The \(\alpha_2\)-isoform has been reported to have much lower affinity for Na\(^+\). The reason for the simultaneous expression of these three subunits, for instance in the hypertrophied heart, is unclear (93). One could speculate that they control different local intracellular Na\(^+\) pools, and there is indeed some evidence that the \(\alpha_1\)-isoform regulates bulk cytosolic Na\(^+\), whereas the other two isoforms may preferentially control [Na\(^+\)]\(c\) close to the Na\(^+\)/Ca\(^{2+}\) exchanger (335).

D. Sarcolemmal K\(^+\) Transporting Proteins Other Than the Na\(^+\)-K\(^+\) Pump

There are few studies on K\(^+\) transporting proteins other than the Na\(^+\)-K\(^+\) pump in heart and skeletal muscle. In rat muscles and in myogenic L6 cells, experiments with \(^{86}\)Rb show a bumetanide-sensitive uptake component (152, 578), and insulin seems to stimulate this uptake (679). Bumetanide and furosemide are diuretics that inhibit the Na\(^+\)-K\(^+\)-Cl\(^-\) transporter. However, in one study, no bumetanide-sensitive \(^{42}\)K uptake could be detected in the rat muscles, suggesting that \(^{86}\)Rb might not be a suitable tracer for measuring bumetanide-sensitive K\(^+\) uptake mechanisms (152) and that skeletal muscle expresses a coupled Na\(^+\) and Cl\(^-\) transport (154). However, Lindinger et al. did not find the same (411) and claim that a Na\(^+\)-K\(^+\)-Cl\(^-\) cotransport exists in rat skeletal muscle (426, 657). Further evidence for the presence of this transporter in skeletal muscle has been provided by identification of an mRNA signal, a protein recognized by specific antibodies and located to muscle cells, and a Cl\(^-\) - and bumetanide-sensitive \(^{86}\)Rb uptake (704). Also, van Mil et al. (647) have observed that the depolarization of skeletal muscle that occurs in hypertonic solution is partly due to intracellular Cl\(^-\) accumulation through activation of a bumetanide-sensitive transport mechanism.

Thus there is accumulating evidence for the presence of a Na\(^+\)-K\(^+\)-Cl\(^-\) cotransport in skeletal muscle that might play a part in controlling both [K\(^+\)]\(c\) and [K\(^+\)]\(v\). In the heart there is good evidence for a Na\(^+\)-K\(^+\)-Cl\(^-\) cotransport that might contribute to volume control and that is activated by aldosterone (158, 427, 473a). Also, activation of furosemide-sensitive K\(^+\) uptake has been observed after elevation of [Ca\(^{2+}\)]\(c\) (367). \(\alpha\)-Adrenergic stimulation increases both K\(^+\) efflux and uptake, probably through activation of the Na\(^+\)-K\(^+\)-Cl\(^-\) cotransport (17, 18, 257, 258). It seems reasonable to conclude that the Na\(^+\)-K\(^+\) pump provides the most important transport mechanism for K\(^+\) uptake in heart and skeletal muscles but that other mechanisms can become activated especially in relation to volume control.

E. Volume Control of Muscle Cells

It is well known that skeletal muscle cells swell at onset of exercise and that swelling is almost linearly related to muscle power at least as judged from the reduction in plasma volume (492, 700). The cellular volume increase can approach 15–20% at maximum (589, 595). Also, it seems that the cells accumulate solute-free fluid (573), which means that the driving force for fluid movement must be an increase of intracellular osmolality and not hydrostatic forces. This was recently corroborated by Ward et al. (669), who showed that \(V_s\) was temporarily reduced after onset of muscle activation, indicating that intracellular osmolytes were the primary driving force for muscle fluid uptake. However, exercise intensity may modulate the relative importance of hydrostatic and osmotic pressures as discussed in section VIII.

Muscle volume changes occur quite quickly during onset of muscle activity (600, 675). Interestingly, it has been shown that hematocrit in the femoral vein increases as rapidly as [K\(^+\)]\(v\) (246). However, the relative rise in hematocrit was almost an order of magnitude smaller than the relative rise of [K\(^+\)]\(c\), and therefore contributed merely by 10–15% to the rise of [K\(^+\)]\(v\). This relationship may be largely coincidental since K\(^+\) and fluid move in opposite directions at onset of muscle activity. The contracting muscle cells swell while they lose K\(^+\) (466, 573, 590). A dissociation in the time course of water and electrolyte fluxes may be explained by the existence of water-only transcapillary channels (674). Furthermore, at cessation of exercise, K\(^+\) is rapidly taken up by the cells, whereas fluid now moves out of the cells and probably accumulates in the interstitial space (573). Complete restoration of the interstitial muscle volume is a much slower process than restoration of muscle cell volume.

On this background it is reasonable to ask what the osmolytes are that cause muscle cells to swell at onset of exercise and does K\(^+\) play any role in muscle cell volume regulation?

Because swelling seems to occur rapidly after onset at all exercise intensities, lactate cannot be the only important osmolyte. At low exercise intensities there is no lactate production, and at high intensities lactate will be inversely related to muscle HCO\(_3^-\) concentration and so have little osmotic activity until [HCO\(_3^-\)]\(c\) is quite low. Also, although changes of other intermediary metabolites...
are small, they may still contribute to the overall increase of osmolytes in proportion to muscle power. However, CrP may be more important (422). There is reasonable evidence to suggest that there is a very rapid muscle power-dependent breakdown of CrP at the onset of exercise so that a new steady-state concentration is achieved (13). Because breakdown of 1 mol CrP will cause accumulation of 1 mol of creatine and 1 mol of Pi, the osmotic effect may be considerable. This explanation fits very well with the findings of Trombitas et al. (642) who showed that during tetanic stimulation fluid accumulates intracellularly especially in the subsarcolemmal space and around mitochondria. Interestingly, it seems that metabolic control is better in slow-twitch muscle than in fast-twitch muscle in the sense that muscle power-dependent initial breakdown of CrP is smaller in the former (13). Hence, one would expect smaller volume changes of slow-twitch muscle. In the heart, metabolic control is very tight, and changes of metabolic fluxes are smaller so that the CrP concentration remains almost constant. Hence, one would predict small or no volume changes of heart muscle with varying work loads.

In situations where the HCO$_3^-$ stores of muscle are depleted, i.e., when intracellular lactate concentration exceeds ~10 mM, lactate will of course contribute to the increase of intracellular osmolytes since it is formed from glycogen. This occurs with high-intensity exercise in skeletal muscle and in both skeletal muscle and heart tissue during ischemia.

In many cells the relationship between volume control and K$^+$ is a very close one (305, 542). In most cells exposure to a hypo- or hyperosmotic medium causes an immediate change of cell volume, which after some minutes is followed by a partial or complete return to almost normal volume. During a regulatory volume decrease (RVD) after a hyposmotic shock, the cells lose KCl and water. During a regulatory volume increase (RVI), which in many cells only occurs when the osmolality of the medium is normalized again after an RVD, the cells take up NaCl through various mechanisms that seem to differ between tissues with subsequent replacement of the Na$^+$ by K$^+$ through the Na$^+$-K$^+$ pump (305, 542). On this background an important question that arises is whether volume changes of skeletal muscle cells are modulated by transfer of K$^+$ during periods of muscle activity.

RVD and RVI, in particular RVD, have been studied in isolated cardiomyocytes and a clonal L6 cell line derived from skeletal muscle. Both RVI and RVD seem to exist in these cells (578, 715, 716). However, in two reports, RVD could only be detected in mature cardiac cells after exposure to catecholamines (158, 668). On the other hand, inhibition of Cl$^-$ channels reduced osmotic cell swelling (114), and isoprenaline treatment caused RVD to appear (668). In another recent investigation, RVD could not be detected in skeletal muscle (494). RVD in cardiomyocytes seems to be associated with activation of stretch-sensitive Cl$^-$ channels (582, 716, 717). However, loss of KCl can account for only a limited fraction of RVD in heart cells, and it seems that loss of amino acids, especially taurine, is also important (530). RVI, on the other hand, is associated with the activation of the Na$^+$-H$^+$ exchange mechanism (578). Interestingly enough, $k_{0.5}$ of the Na$^+$-K$^+$ pump for [Na$^+$]$_c$ is decreased in isolated cardiac myocytes exposed to hypotonic media, whereas it is increased when cells are shrunk by hyposmotic media (692). This can explain why cell shrinkage leads to a sustained elevated [Na$^+$]$_c$ in these cells, whereas during swelling [Na$^+$]$_c$ is reduced. How important these mechanisms are for normal muscle and heart function remains unclear, since both RVD and RVI take several minutes to develop (716). However, with prolonged exercise and in pathological conditions like ischemia, they may be important.

Interestingly, further evidence that K$^+$ is not essential for cell volume control in muscle cells is suggested by the fact that there was an almost negligible swelling of Purkinje fibers where all intracellular K$^+$ was replaced with Na$^+$ (574). Furthermore, during reactivation of the Na$^+$-K$^+$ pump which restores high intracellular K$^+$ levels, shrinkage of the cells was minimal in these experiments (575). Thus, as long as K$^+$ movements are linked to opposite movements of Na$^+$, which is the case for all electrical activity, the contribution of K$^+$ to volume control seems to be minimal.

Although movements of K$^+$ do not seem important for control of muscle volume, changes of muscle cell volume will of course affect intracellular concentrations as outlined above. Thus exercise-induced muscle swelling will under normal conditions be large in fast-twitch muscle, and almost not existent during increased work load in the heart. Indeed (183), whereas in heart muscle the expected rise in [Na$^+$]$_c$ is sufficient to explain the activation of the Na$^+$-K$^+$ pump (see above), the swelling-induced attenuation of the rise of [Na$^+$]$_c$ might contribute somewhat to the less efficient activation of the Na$^+$-K$^+$ pump in the EDL muscle compared with the soleus muscle (183). Also the accentuated fall in [K$^+$]$_c$ in cells that swell will contribute to the depolarization.

IV. POTASSIUM IN ELECTRICAL EVENTS OF MUSCLE

A. Membrane Potential

1. Role of K$^+$

The most important role of K$^+$ for striated muscle function is to determine the $E_{\text{m}}$, which is the basis for excitability. This role rests on two principal conditions. First, the [K$^+$]$_c$ must be kept high (100–160 mM) while at
the same time $[K^+]_i$ must be in the range of 4 mM. Second, the cell membrane permeability to $K^+$ must be considerably higher than to other cations. The focus of this review is on the effects of $K^+$ transfer between intra- and extracellular compartments of heart and skeletal muscle especially during activation and in some pathological conditions. The interest in this topic arises from the possible detrimental effects of such changes on excitability. A number of questions also arise, such as under which exercise or electrical stimulation conditions is the concentration gradient for $K^+$ across the sarcolemma perturbed to such an extent that excitability is compromised, and do permeability changes occur that affect the role of $K^+$ in excitability? Of special concern is the interaction of $K^+$ with $Cl^-$, especially during periods of net ion transfer across the sarcolemma. As a background for this review we give a brief description of the factors that govern resting $E_m$ and the AP.

In the resting steady-state condition at 37°C with a $[K^+]_o$ of ~5 mM, $E_m$ is ~80 mV in fast-twitch (81, 84, 159, 269, 321, 429, 438, 451, 452, 583, 618, 714) and ~73 mV in slow-twitch (8, 81, 84, 106, 159, 269, 429, 438, 451, 480, 618, 714) skeletal muscles from rodents, whereas it was recorded at ~81 mV both in Purkinje fibers (217, 475, 574, 580) and ventricular cells (37, 89, 143, 356, 396, 455, 481, 547, 699) from various mammalian species including human tissue.

In the steady-state situation the $E_m$ is well described by the Goldman-Hodgkin-Katz (GHK) equation

$$E_m = \frac{2.303 \times RT}{F} \log_{10} \left( \frac{[K^+]_i + \alpha [Na^+]_i + \beta [Cl^-]_i}{[K^+]_o + \alpha [Na^+]_o + \beta [Cl^-]_o} \right)$$

where $R$ is the gas constant, $T$ is the absolute temperature, $F$ is the Faraday constant, and 2.303$RT/F$ equals ~61.5 mV at 37°C, with $\alpha$ and $\beta$ being the ratios of the permeability coefficients for Na$^+$ and Cl$^-$, respectively, to K$^+$ (303). The $\alpha$ value is in the order of 0.01 (64, 81, 84, 106, 134, 394, 585). In resting human skeletal muscle, the conductance to Cl$^-$ is three times higher than to K$^+$ (381). This means that $\beta$ can be large. $[K^+]_o$, $[K^+]_i$, $[Na^+]_o$, $[Na^+]_i$, $[Cl^-]_o$, and $[Cl^-]_i$, are the concentrations of K$^+$, Na$^+$, and Cl$^-$ inside the cell in the cytosol (c) and at the cell surface (s) (see, e.g., Ref. 718). This steady-state description of the $E_m$ has been a very fruitful approximation but does not, however, take into account three important aspects. First, the steady-state condition rarely prevails in muscle. Any changes in the beating frequency of the heart or stimulation rate of muscle is accompanied by net ion transfer across the cell membrane, and such changes occur all the time. This topic is treated extensively later (see sect. v). Second, the permeability of the membrane to K$^+$ (or Na$^+$) is not constant ($\alpha$ is not constant), for instance, when $[K^+]_i$ varies. Third, electrogenic ion transporters create currents that contribute to the $E_m$ particularly under non-steady-state conditions (21, 145, 525).

Several authors omit Cl$^-$ from the equation. This can be done if the membrane permeability to this anion is either very high (large $\beta$) or almost nil (very small $\beta$). If $\beta$ is high, and Cl$^-$ is entirely passively distributed across the membrane, Cl$^-$ will distribute according to the potential determined by K$^+$ and Na$^+$. This has been thought to be the case in skeletal muscle. If this is not the case and Cl$^-$ is not in equilibrium across the membrane, either in the steady state or during transient changes of other electrolytes, Cl$^-$ may contribute to the potential.

2. Observations on $K^+$ dependence of $E_m$

There are several classic reports on skeletal muscle where the steady-state $E_m$ was measured at various extracellular K$^+$ concentrations (see, for instance, Refs. 66, 300). These conclude that the skeletal muscle membrane behaves primarily as a K$^+$ electrode. The introduction of ion-selective microelectrodes some 25 years ago made it possible to determine in more detail the difference between $E_m$ and $E_K$ and other ions, especially Cl$^-$. These electrodes reflect the ion activities and allow for continuous registration of changes in intracellular ion activities when the extracellular medium is changed. These studies basically confirm that the sarcolemma of both skeletal muscle and heart muscle behave as K$^+$ electrodes at extracellular concentrations above ~4 mM (208, 292, 475). Therefore, during increases in $[K^+]_o$ from its normal level, the membrane will depolarize by somewhat less than 18 mV for a doubling of the concentration in the steady state (80, 81).

However, when $[K^+]_o$ is reduced below 4 mM, the GHK equation predicts deviation from behavior of a pure K$^+$ electrode due to the small permeability to Na$^+$. Interestingly, in some studies both in heart and skeletal muscle, even more positive potentials have been described. In fact, below 3–2.5 mM $[K^+]_o$ a stable $E_m$ 20–60 mV positive to that predicted by the GHK equation has been observed (88, 217, 397, 583, 714). Some cells exhibit two stable resting potentials in this region of $[K^+]_o$, whereas other cells can switch from one to the other (217, 397, 583, 584, 696). This is partly because lowering of $[K^+]_o$ will reduce the conductance of the inward rectifier (129, 269, 291, 398, 446, 551, 584) (see sect. vi).

This depolarizing effect of low $[K^+]_o$ is not observed in all preparations or cells (291, 300), and there seems to be considerable variation from preparation to preparation or between cells. This may reflect different channel densities or different sensitivities of the gating mechanism of the channels to the electrochemical K$^+$ gradient.

Some interesting observations should be mentioned here. Mølgaard et al. (480) reported a dichotomy of re-
corded \( E_m \) in rat soleus muscle at \([K^+]_o\) below \(-3\) mM, one population being 30 mV more positive than the other at 1 mM \([K^+]_o\). Recordings were made in the steady-state condition after 20-min equilibration. The authors ascribed the phenomenon to the presence of some fast-twitch fibers on the surface of this slow-twitch muscle, but they did not identify the impaled cells, and the difference is much larger than ordinarily observed for soleus compared with EDL (see above). It is very probable that the dichotomy reflects the bistability of the \( E_m \) described above. McCullough et al. (455) observed that in multicellular specimens from diseased human ventricles there was a population of depolarized cells that hyperpolarized when \([K^+]_o\) was raised from 4 to 7 mM. \([K^+]_c\) did not change, and activation of the electrogenic Na\(^+\)-K\(^+\) pump could be ruled out, indicating that the reason was most likely to be low K\(^+\) permeability at 4 mM. The sensitivity of the IRK channel to extracellular K\(^+\) had apparently shifted. Although an increased Na\(^+\)-K\(^+\) pump rate could be ruled out when \([K^+]_o\) was high, it is still possible that \([Na^+]_c\) was higher than normal in these cells. Matsuda (445) recently showed in heart muscle that the IRK was inhibited at elevated \([Na^+]_c\). Provided this is a general phenomenon it could be a mechanism for depolarization of muscle cells at normal \([K^+]_o\) and would contribute to reduced excitability during exercise.

In conclusion, normally the resting \( E_m \) of heart and skeletal muscle cells can be ascribed to the K\(^+\) gradient and the high resting sarcolemmal permeability to K\(^+\). The cells depolarize as expected for a K\(^+\) electrode, especially when \([K^+]_o\) is raised above normal. On the other hand, there is a variable response to moderate reductions of \([K^+]_o\) whereby some cells will hyperpolarize while others will be little affected. In pathological conditions with pronounced hypokalemia or in conditions where permeability to K\(^+\) is reduced, the cells of both tissues may depolarize to the level of inexcitability or to levels of spontaneous electrical activity. This behavior may be ascribed to the properties of the IRK channel.

In the intact, perfused, exercising muscle one should keep in mind that not only does \([K^+]_o\) rise but that there is also a dissipation of intracellular K\(^+\) and a cell swelling that may cause substantial lowering of \([K^+]_c\). (26, 329). Thus the ratio of \([K^+]_o\) to \([K^+]_c\) may easily be more than doubled in some circumstances.

3. Contribution of Cl\(^-\) to \( E_m \)

It is a classic question whether or to what extent Cl\(^-\) contributes to the \( E_m \). It was recognized quite early that skeletal muscles have higher permeability to Cl\(^-\) than to K\(^+\) and their \( E_m \) is therefore to a large extent a Donnan equilibrium potential (4, 300, 311, 381). The basic criterion for a Donnan equilibrium is that the concentrations of K\(^+\) and Cl\(^-\) will adjust so that the product of the concentrations of the two ions is equal on the inside and the outside of the membrane. The concept of the Donnan equilibrium in skeletal muscle was developed by Boyle and Conway (66) and implies that the \( E_m \) equals the equilibrium potentials for both K\(^+\) and Cl\(^-\), or with some allowance for the contribution of other cations, especially Na\(^+\), as outlined above, that at least Cl\(^-\) is distributed according to the \( E_m \).

Indeed, in skeletal muscle, the equilibrium potential for Cl\(^-\) is very close to the \( E_m \), in the steady-state condition (6, 59, 148, 394, 452, 651). However, there seems to be an inward transport of Cl\(^-\) by means of the Na\(^+\)-K\(^+\)-Cl\(^-\) cotransporter (7). The basis for this is that intracellular [Cl\(^-\)] \(_c\) is 1–2 mM higher than predicted by the \( E_m \). Hence, Cl\(^-\) has a slight depolarizing effect on the cells. The addition of the Cl\(^-\) channel blocker 9-anthracene carboxylic acid (9-AC) caused a rapid rise in [Cl\(^-\)] \(_c\), showing that in the resting condition there was a Cl\(^-\) uptake balanced by an outward Cl\(^-\) flux that was inhibited by the drug (7).

The high Cl\(^-\) conductance stabilizes the \( E_m \) in normal muscle. In myotonia caused by mutations of the CLC-1 Cl\(^-\) channel there is increased excitability, and firing of the muscle does not cease when the motoneuron stops firing (71, 209). Hodgkin and Horowicz (300) elegantly showed that the effect of Cl\(^-\) on \( E_m \) is transient after sudden changes in [K\(^+\)] \(_o\). When concentration changes are made so that [K\(^+\)] and [Cl\(^-\)] vary inversely while the concentration product remains constant, \( E_m \) changes are very rapid and remain stable since no net transfer of the two ions across the membrane takes place. By changing [K\(^+\)] \(_o\) alone one sees an instantaneous change of the \( E_m \) followed by a slower change, due to diffusion of Cl\(^-\) across the sarcolemma, until equilibrium is again attained. This was confirmed by Dulhunty (159) in mammalian muscle, although the time course of the second phase was more rapid than in the amphibian muscle. Interestingly enough, the same study showed that the diaphragm and the intercostal muscles do not share this property with the other skeletal muscles. That sudden changes in [K\(^+\)] \(_o\) lead to slower changes in [Cl\(^-\)] \(_o\) has been directly measured with chloride-selective microelectrodes (7, 452, 651). During and after high-intensity exercise there are rapid changes of [K\(^+\)] \(_o\) as described below. The effect of these changes on the \( E_m \) may be very much affected by how fast Cl\(^-\) redistributes across the membrane. Despite a rapid accumulation of K\(^+\) in the extracellular space, the cells may remain polarized for periods of several seconds or even minutes due to this stabilizing effect of Cl\(^-\) on the \( E_m \).

One view held is that heart muscle, in contrast to skeletal muscle, has a low permeability to Cl\(^-\) and that the \( E_m \) is basically a K\(^+\) diffusion potential (88, 208, 312). In line with this, [Cl\(^-\)] \(_o\) is three to four times higher than predicted from the \( E_m \) (38, 78, 143, 649), and there is also
a Cl\textsuperscript{-} uptake in exchange for HCO\textsubscript{3}\textsuperscript{-} (650, 652). However, it has recently become clear that resting heart muscle is probably quite different from beating heart muscle. Both catecholamines and cell swelling or stretch will induce a high Cl\textsuperscript{-} permeability (2, 267, 268, 716, 717). Hence, during normal heart activity, the background Cl\textsuperscript{-} conductance may be quite high, and, as in skeletal muscle, the effect of sudden changes in [K\textsuperscript{+}]\textsubscript{s} on \(E_m\) will probably be attenuated.

It seems reasonable to conclude that Cl\textsuperscript{-} does in fact stabilize the \(E_m\) in most skeletal muscles and probably also the heart but that this effect is dynamic in the sense that the effect of Cl\textsuperscript{-} will only be transient until the net flux of Cl\textsuperscript{-} across the sarcolemma has ceased and Cl\textsuperscript{-} again is close to equilibrium. One would expect this effect to be most prominent immediately after changes in firing frequency that cause variations in [K\textsuperscript{+}]\textsubscript{s}. Evidence in favor of this is "nature's own experiment" with myotonia along with experiments in which Cl\textsuperscript{-} was substituted with impermeant anions or where Cl\textsuperscript{-} channels were inhibited with 9-AC (118).

4. Electrogenic ion transport

Because the stoichiometry of the Na\textsuperscript{+}-K\textsuperscript{+} pump is 3Na\textsuperscript{+}:2K\textsuperscript{+}, one net charge is carried per cycle; meanwhile, the pump generates a current, the size of which is determined by the pump rate. The pump is not an ideal current generator since it is sensitive to the \(E_m\) (see sect. iii).

The net outward Na\textsuperscript{+}-K\textsuperscript{+} pump-induced current must be balanced by a corresponding inward current that closes the circuit. It is the resistance to this inward current that actually determines the contribution of the pump to \(E_m\). In the steady-state condition of resting cells, where intracellular ion concentrations and \(E_m\) are stable, not only are the currents in balance, but also the net transfer of ions by the pump remain balanced. This means that inward pumping of two K\textsuperscript{+} is counterbalanced by the outward flux of two K\textsuperscript{+}. The three transported Na\textsuperscript{+} are naturally also in balance. This means that the net charge of the electrical circuit is in fact carried by one Na\textsuperscript{+} per pump cycle in the resting situation as pointed out by Boyden et al. (64) and illustrated in Figure 5, top panel. The effect is a hyperpolarization that varies from a few millivolts to almost 10 mV depending on pump density and rate (64, 106, 394, 563). Due to the reduced Na\textsuperscript{+}-K\textsuperscript{+} pump rate at low [K\textsuperscript{+}]\textsubscript{s}, the pump contribution to \(E_m\) diminishes as [K\textsuperscript{+}]\textsubscript{s} is lowered below 4 mM (see below) contributing to the curved nature of the relationship between \(\log_{10} [K^+]_s\) and \(E_m\). There are a number of different pathways for the inward Na\textsuperscript{+} current. The Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger carries three Na\textsuperscript{+} for one Ca\textsuperscript{2+} so that it ideally matches the Na\textsuperscript{+}-K\textsuperscript{+} pump by also carrying an extra charge. Because there are other Na\textsuperscript{+} exchangers, and also a basal permeability to Na\textsuperscript{+}, the resistance cannot be ascribed to one single pathway for Na\textsuperscript{+}.

The almost instantaneous depolarization seen when the pump is suddenly blocked by digitalis reflects that this Na\textsuperscript{+} circuit is broken. Thereafter, as the ionic gradients dissipate, the cell depolarizes further (106, 563). This

![Diagram showing electrogenic transport by the Na\textsuperscript{+}-K\textsuperscript{+} pump during steady-state conditions.](http://physrev.physiology.org/)

FIG. 5. Rheogenic transport by the Na\textsuperscript{+}-K\textsuperscript{+} pump during steady-state conditions (top) when the small "leak" of Na\textsuperscript{+} and K\textsuperscript{+} is exactly balanced by the pump, and the current generated by the pump contributing to \(E_m\) is small and is carried by Na\textsuperscript{+} (dotted line represents the charge circuit driven by the pump). Only one of the Na\textsuperscript{+} carried by the pump will be part of this circuit. During activity, an additional electric circuit is established by the action potential (middle) carrying Na\textsuperscript{+} into the cell and K\textsuperscript{+} out of the cell (stippled-dotted line). After onset of activity, this current will exceed the Na\textsuperscript{+}-K\textsuperscript{+} pump capacity by a number \(n (n + 2 > \text{pump rate})\), and the cell will gain Na\textsuperscript{+} and release K\textsuperscript{+}. Still the pump contribution to \(E_m\) may not be large due to the high conductance for the one Na\textsuperscript{+} that is still part of the charge circuit corresponding to the net charge carried by the pump. During recovery (bottom), the Na\textsuperscript{+}-K\textsuperscript{+} pump will reestablish the normal resting intracellular ion composition, and its contribution to \(E_m\) now derives from an electric circuit in which K\textsuperscript{+} carries the inward current and is taken up by the cell through the classic inward rectifier IRK. Thus the contribution to \(E_m\) is now mainly determined by the conductance to K\textsuperscript{+}. 
latter phenomenon is not usually regarded as the Na\(^{+}\)-K\(^{+}\) pump contribution to the \(E_m\).

The situation may be entirely different when there has been a perturbation of the intracellular environment, for instance, after a burst of AP or after a period of ischemia, especially in the heart. In this case the Na\(^{+}\)-K\(^{+}\) pump will restore the Na\(^{+}\)-K\(^{+}\) balance of the cell, along with a net transfer of Na\(^{+}\) and K\(^{+}\) across the sarcolemma. Still, the laws of electricity cannot be obviated so the electric circuit must be complete. The basic feature is that pump rate is elevated, possibly due to several mechanisms as discussed later, but elevated [Na\(^{+}\)]\(_c\) is an important part of it. This increased outward pump current will hyperpolarize the cell (632). This was first observed by Ritchie and Straub (538) and then by Rang and Ritchie (525) in unmyelinated nerves and by Thomas (631) after injection of Na\(^{+}\) into snail neurons. This hyperpolarization will reduce the driving force for outward K\(^{+}\) diffusion so that K\(^{+}\) accumulates in the cell. The hyperpolarization may even reach values more negative than \(E_K\) (8, 68).

In this case the electric charge that closes the circuit may be carried by K\(^{+}\) (Fig. 5, bottom panel). Especially if [K\(^{+}\)]\(_c\) is raised there will be low resistance to inward K\(^{+}\) diffusion through the IRK channel. Even other ions, for instance, outward diffusion of Cl\(^{−}\), can also carry the required charge (8, 526).

Several interesting aspects of this dynamic situation become evident. First, the hyperpolarization of the cells caused by the Na\(^{+}\)-K\(^{+}\) pump may affect excitability. Second, the apparent ratio between cellular gain of K\(^{+}\) and loss of Na\(^{+}\) may vary between 1 and very low values even though the pump stoichiometry is 2:3. It is probably not correct that the variable ratio between gain of K\(^{+}\) and loss of Na\(^{+}\) reflects a variable stoichiometry of the pump as claimed by some investigators (443).

The hyperpolarizing ability of the Na\(^{+}\)-K\(^{+}\) pump is illustrated in Figure 6. When [Na\(^{−}\)]\(_c\) is high after a procedure that causes Na\(^{+}\) uptake into the Purkinje fibers, reactivation of the Na\(^{+}\)-K\(^{+}\) pump causes the \(E_m\) to become transiently more than 75 mV negative to \(E_K\). It should be noted that in this particular experiment \(E_K\) is quite high since [K\(^{+}\)]\(_c\) was 30 mM. When the procedure was carried out in CI\(^{−}\)-free medium and in the presence of Ba\(^{2+}\) (a blocker of several K\(^{+}\) channels), activation of the Na\(^{+}\)-K\(^{+}\) pump by Rb\(^{+}\) increased the hyperpolarization to almost −150 mV, thus illustrating that inward K\(^{+}\) current through the IRK balanced most of the pump current. Accordingly, because K\(^{+}\) uptake was also occurring through these channels by means of the favorable inward electrochemical gradient, the ratio between gain of intracellular K\(^{+}\) and loss of Na\(^{+}\) was 0.86, which is higher than the coupling ratio of the Na\(^{+}\)-K\(^{+}\) pump (574).

In a more physiological setting hyperpolarization to more negative values than \(E_K\) was observed by Rasmussen and co-workers (528, 529). This was recorded in right atrial specimens from patients. After incubation in the cold, rewarmin caused a digitalis-sensitive transient hyperpolarization. During this period exposure to acetylcholine which opens the acetylcholine-sensitive K\(^{+}\) channels caused a depolarization and not the usual hyperpolarization, indicative of a pump-induced potential negative to \(E_K\). Also, Ba\(^{2+}\) potentiated the hyperpolarization, in line with an inward K\(^{+}\) current through the IRK.

Similarly, Hicks and McComas (286) showed that in intact soleus muscles of anesthetized rats, after repetitive tetanic contractions for 5 min, \(E_m\) became more negative by −10 mV. Possibly this is a conservative estimate of the true difference between \(E_m\) and \(E_K\) in these muscles.

![Figure 6. Demonstration of the hyperpolarizing effect of the Na\(^{+}\)-K\(^{+}\) pump. Sheep cardiac Purkinje fibers were loaded with Na\(^{+}\) as described by Sejersted et al. (574). The Na\(^{+}\)-K\(^{+}\) pump was activated by addition of 30 mM K\(^{+}\) or Rb\(^{+}\). Top: calculated equilibrium potential for K\(^{+}\) (\(E_K\)) after addition of K\(^{+}\). Note that in this situation [K\(^{+}\)]\(_c\) is initially close to zero so that \(E_K\) will be positive at the start. The solid line shows the voltage response during activation of the Na\(^{+}\)-K\(^{+}\) pump by K\(^{+}\). The dotted line shows the voltage response after a second Na\(^{+}\) loading of the same fiber when the Na\(^{+}\)-K\(^{+}\) pump was again activated, but now with Rb\(^{+}\) in the presence of 0.7 mM Ba\(^{2+}\). Note that the Na\(^{+}\)-K\(^{+}\) pump hyperpolarizes the cell almost −150 mV at a time when \(E_K\) is close to zero. Bottom: the 2 solid lines show the decline of intracellular Na\(^{+}\) activity and the concomitant rise of intracellular K\(^{+}\) activity as simultaneously recorded by Na\(^{+}\)- and K\(^{+}\)-selective microelectrodes during K\(^{+}\) activation of the pump. The dotted line shows the decline of intracellular Na\(^{+}\) activity corresponding to the much more pronounced hyperpolarization obtained by activating the Na\(^{+}\)-K\(^{+}\) pump by Rb\(^{+}\) in the presence of Ba\(^{2+}\). The rate of recovery of intracellular Na\(^{+}\) activity was almost the same as during activation of the Na\(^{+}\)-K\(^{+}\) pump by K\(^{+}\) despite the markedly enhanced voltage response (Sejersted, unpublished data).](http://physrev.physiology.org/Downloaded from http://physrev.physiology.org/)
since there might have been an accumulation of $K^+$ in the interstitium. The response was sensitive to ouabain, and hence, the $Na^+-K^+$ pump by hyperpolarizing the cell both speeds up reaccumulation of $K^+$ and prevents the muscle from becoming inexcitable due to a $K^+$-induced depolarization.

The same phenomenon may explain why catecholamines can restore contraction force in muscle made inexcitable by exposure to increased $[K^+]_e$ (101). Catecholamines stimulate the $Na^+-K^+$ pump as described above and thereby create a transient hyperpolarization associated with a transient net flux of $K^+$ and $Na^+$ across the sarcolemma. The effect is transient, probably because the fall of $[Na^+]_c$ will eventually counterbalance the effect of the catecholamines on the $Na^+-K^+$ pump.

Thus restoration of the cellular ionic balance after periods of $Na^+$ loading and $K^+$ loss is achieved by activation of the $Na^+-K^+$ pump, which in turn causes cellular hyperpolarization so that an extra $K^+$ is taken up through the inward rectifier.

B. Excitability

Excitability is defined by the amount of inward current (rheobase) that is required to depolarize the cells sufficiently to reach the threshold potential that triggers opening of enough $Na^+$ channels to elicit an AP. Hence, $K^+$ is important for excitability in two aspects. First, if the basal conductance to $K^+$ is very high, a large current is needed to depolarize the cells since outward movement of $K^+$ will short circuit the inward current. Therefore, opening of ATP or acetylcholine-sensitive $K^+$ channels, for instance, may render muscle and heart inexcitable. This is probably the case only in pathological conditions, such as severe ischemia. However, exhausted skeletal muscle fibers have a greatly increased background conductance possibly due to activation of $Ca^{2+}$-activated $K^+$ channels (197, 435). Second, through its major role in maintaining the resting $E_m$, $K^+$ determines both the fraction of inactivated $Na^+$ channels and how close the resting $E_m$ is to the threshold potential.

Thus a rise of $[K^+]_e$ will have a complex effect on excitability. $K^+$ conductance will rise through the effect on IKK channels, and the effect on $E_m$ is variable as outlined above, although a depolarization occurs above 4 mM. First, this depolarization may in theory increase excitability since the distance to the threshold potential becomes smaller, but eventually inactivation of $Na^+$ channels may dominate.

Complete inexcitability may therefore be the result of two entirely different mechanisms. Too much current may be required to reach threshold, or too many $Na^+$ channels may be in the inactivated state. So far failure at the neuromuscular junction due to too little current through the acetylcholine receptor has not been reported as a factor in fatigue, although reduced rate of rise of the nerve AP during hyperkalemia will in fact reduce release of acetylcholine. In pathological states like myasthenia gravis this is the site of failure. Meanwhile, there has been considerable focus on the slow inactivation of $Na^+$ channels in skeletal muscle cells. With a declining number of channels in the active state, AP amplitude will gradually be reduced until the $Na^+$ current becomes too small to be able to cause regeneration. This is the classic depolarization block or cathodal block of excitation. Also, $Na^+$ channel inactivation will cause failure of initiation of AP at the neuromuscular junction (705). The question one asks in this context is whether perturbations of $K^+$ distribution with exercise are large enough to affect excitability in skeletal muscle or heart.

Slow inactivation of the $Na^+$ current was first described by Hodgkin and Huxley (302). Recently, the molecular basis for this property has been identified in cloned $Na^+$ channels, but thanks to new patch-clamp techniques there has also been a renewed interest in the electrophysiological aspect of this property of the $Na^+$ channels. Notably Ruff and co-workers (543, 545, 546) concluded that with regard to the voltage sensitivity of the inactivation process of $Na^+$ channels there are important differences between fast- and slow-twitch fibers. The $E_m$ at which 50% of the channels are inactivated is quite close to the resting $E_m$ in fast-twitch fibers of both rat and humans. Despite their more positive resting potential, slow-twitch fibers displayed relatively less inactivation than fast-twitch fibers. This may be why generation of AP during current injection occurs with a smaller depolarization in rat soleus muscles compared with EDL (705). However, these results were obtained at 20°C, and Ruff (544) recently reported that increasing the temperature to 37°C shifted the $E_m$ at which 50% of the $Na^+$ channels were inactivated +16 mV. Thus 99% of the $Na^+$ channels of the fast-twitch fibers became excitatable. Even so, the results indicate that fast-twitch fibers might be quite sensitive to depolarization caused by an altered $K^+$ gradient, but that on the other hand hyperpolarization caused by the $Na^+-K^+$ pump might efficiently restore or maintain $Na^+$ channels in the excitatable state. Interestingly, more current is needed to reach threshold in fast-twitch compared with slow-twitch fibers (161).

Juel (330) showed that at 10 mM $[K^+]_e$ 21 and 28% of fibers in the mouse soleus and EDL muscles, respectively, were inexcitable at an $E_m$ of −65 mV, whereas at a $[K^+]_e$ of 15 mM excitation was completely blocked. In frog muscle, Renaud and Light (536) found similar results. Recently, Cairns and co-workers (80, 81) examined the relationship between $E_m$ and contractile force during exposure to various $[K^+]_e$ in both soleus and EDL muscles from rats and mice. Complete inexcitability was seen at 12 mM in the soleus muscles of both species and in the EDL of
mice, but 14 mM was required to block excitation in the rat EDL. The effect of elevated [K\(^+\)]\(_s\) occurred slowly with a time constant of almost 40 min. This indicates that restricted diffusion of either K\(^+\) or Cl\(^-\) slowed the depolarization of these intact, isolated muscles and fiber bundles. When the Na\(^+\)-K\(^+\) pumps had been inhibited by ouabain, the effect of elevated [K\(^+\)]\(_s\) on contractile force occurred faster, which could mean that with intact Na\(^+\)-K\(^+\) pumps the K\(^+\) entering from the muscle surface was taken up by the cells on its way into the muscle, thus slowing the rate of rise of [K\(^+\)]\(_s\). At room temperature the \(E_m\) values when all fibers were inexcitable were \(-54\) and \(-57\) mV in the soleus and EDL muscles, respectively. The differences between fast- and slow-twitch fibers are therefore much more subtle than anticipated from the Na\(^+\) channel inactivation characteristics.

During fatiguing stimulation of isolated muscles, Juel et al. (329) reported \(E_m\) of about \(-57\) mV in both soleus and EDL. Similar depolarizations have been observed with continuous tetanic stimulation of frog fibers (386, 387). More moderate depolarization has been reported in frog muscles during intermittent stimulation (27, 688). Significantly, these muscles were not perfused. It is therefore important to remember that in such experiments, as well as those in which [K\(^+\)]\(_s\) is increased, the Na\(^+\)-K\(^+\) pump might not necessarily have contributed to the \(E_m\) to prevent depolarization by the same amount as in vivo. Taken together, we conclude that the perturbations seen during exercise may depolarize some muscle fibers sufficiently to cause complete excitation block.

C. Action Potential

The AP differs greatly in skeletal muscle and in the heart. In skeletal muscle it is short-lasting, taking only a few milliseconds as in neurons, whereas in the heart in most species it is much more prolonged and can last \(>600\) ms. There are also important differences in the AP between fast- and slow-twitch muscle and between different regions of the heart, even within the ventricles (98, 165, 166, 259, 309, 673). The most important reasons for these differences are the K\(^+\) currents that terminate the AP and, in addition, variable transient outward K\(^+\) currents in heart cells. However, although the length of the AP is related to the duration of the K\(^+\) currents (see sect. vi), it is the timing of channel opening that is more important. For instance, toward the end of the plateau of the AP of ventricular cells very little current is passed in either direction across the sarcolemma since few channels are open.

To understand the relationship between electrical activity in skeletal muscle or heart and the release of K\(^+\) it is important to note that inward currents and outward currents must be matched although there may be some time delays of outward currents. The inward currents are carried by Na\(^+\) and in the heart also by Ca\(^{2+}\), whereas the outward currents are carried by K\(^+\). In addition, Cl\(^-\) may carry both outward and inward currents. If there is little background conductance and no overlap in time between inward and outward currents, then the height of the AP is directly related to the amount of current that is passed. This may be the case, for instance, in skeletal muscle, provided that opening of Na\(^+\) channels is concurrent with closure of the inward rectifier and delayed rectifiers do not open until the Na\(^+\) channels have closed. However, there will usually be some conductance for inward and outward currents simultaneously, for instance, the high Cl\(^-\) conductance in skeletal muscle. Because fast inactivation of Na\(^+\) channels is voltage dependent, they will stay open for a longer period if the amplitude of the overshoot of the AP is reduced such that more inward current may be passed. However, the peak Na\(^+\) current is so large compared with other currents that this effect is probably very small. Therefore, the theoretical minimal current required to elicit an AP may not be far from the one actually measured. With a depolarization of 100 mV and a capacitance of 1 \(\mu F/cm^2\) (0.01 F/m\(^2\)) the required ion flux will be \(10^{-8} \text{ mol \cdot AP}^{-1} \cdot \text{m}^{-2} (1 \text{ pmol/cm}\(^2\)).\) Capacitance has been reported in the range of 4–6 F/kg, and predicted flux of both Na\(^+\) and of K\(^+\) will then be 4–6 nmol \(\cdot \text{AP}^{-1} \cdot \text{g wet wt}^{-1}\) in skeletal muscle.

In the present context, again the question is whether perturbations of K\(^+\) are large enough to affect the AP. Several investigators have reported reduced amplitude and broadening of the AP in unfatigued fibers during elevated [K\(^+\)]\(_s\) (325, 330, 368, 369, 386). Similar AP changes have been reported with fatigue during repeated stimulation (27, 259, 330, 368, 369, 386, 387, 434). The broadening of the AP is associated with reduced propagation velocity which is most pronounced in fast-twitch fibers compared with slow-twitch fibers (330, 369). Because the Na\(^+\) current is so large compared with other currents, the amplitude is first of all determined by the number of Na\(^+\) channels that open. The two questions that arise are whether these AP changes with fatigue are related to the ionic shifts that occur, and whether these ionic shifts are of sufficient magnitude to cause reduced force of contraction?

Oevergaard et al. (512) recently examined the relationship between variations in the Na\(^+\) and K\(^+\) gradients across the sarcolemma and the compound action potential (M wave) in isolated rat soleus muscles. They point out that in addition to the number of open Na\(^+\) channels, the Na\(^+\) gradient across the sarcolemma will determine the amplitude of the AP. Provided [Na\(^+\)]\(_s\) is doubled (as illustrated in Fig. 4) during activity the driving force for Na\(^+\) will be reduced by almost 20 mV. They mimicked the rise of [Na\(^+\)]\(_s\) by reducing [Na\(^+\)]\(_s\) from 140 to 85 mM. Combined with an elevation of [K\(^+\)]\(_s\) to 9 mM, the M-wave
are not usually metabolically exhausted (see sect.IX), and plain decreased AP amplitude. However, fatigued fibers metabolically exhausted muscle fibers (197, 435) could indeed, the very high membrane conductance seen in metabolism might be related to metabolic factors (434). In fact, the very high membrane conductance seen in fatigue might be related to metabolic factors (434). In certain cases, the high membrane conductance might be related to metabolic changes of intra- and extracellular electrolytes, and the M-wave area was closely correlated with changes of force of contraction (see sect. IXB2).

Initial investigations indicated that AP changes with fatigue might be related to metabolic factors (434). Indeed, the very high membrane conductance seen in metabolically exhausted muscle fibers (197, 435) could explain decreased AP amplitude. However, fatigued fibers are not usually metabolically exhausted (see sect. IX), and especially the hyperpolarization observed by some investigators immediately after muscle activation is not reconcilable with a very high membrane conductance. More recent studies have therefore concluded that the AP fatigue is probably the result of ion shifts that depolarize the cells during exercise (26, 27). Balog and Fitts (26) point out that there is a close temporal relationship between recovery of ion gradients, $E_{m}$, and force.

Interestingly, inhibition of K$_{ATP}$ channels caused a prolongation of the AP both in unfatigued and fatigued frog fibers (405), indicating that this channel also normally contributes to repolarizing K$^{+}$ currents. There is some evidence that K$^{+}$ release via these channels may contribute to fatigue (164).

We therefore conclude that in skeletal muscle, K$^{+}$ shifts close to those seen with high intensity exercise (see sect. VIII) may cause significant depolarization, but that in vivo probably both Cl$^{-}$ and the Na$^{+}$-K$^{+}$ pump will attenuate this effect. With sufficient depolarization both failure of AP initiation and propagation occur that may cause reduced force of contraction. However, several authors report that the changes in the AP seen during intensive skeletal muscle stimulation is not of sufficient magnitude to explain the reduced force of contraction (201). They therefore conclude that the cause of contractile failure must reside distal to the surface membrane, possibly in the t-tubular structure (27, 387, 470, 513).

D. Role of T Tubules

Contraction is prevented in muscle fibers that have been detubulated by glycerol treatment (222). The reason for this is that the voltage sensor (dihydropyridine receptor) is located in this tubular structure in close juxtaposition to the Ca$^{2+}$ release channel of the sarcoplasmic reticulum. With this taken into consideration, a discussion of fatigue related to membrane events must necessarily include a discussion of the role of the t tubules in excitation.

Detubulated skeletal muscle fibers can still propagate AP in the surface membrane, but the AP do not show the early afterpotential that is typical of skeletal muscle (222, 387). This is taken to indicate that propagation of the AP into the t-tubular structure is absent. Propagation of the AP in the t-tubular system has yet to be directly shown, although there is considerable indirect evidence (47, 373). In a series of experiments, Heiny et al. (275) and Heiny and Vergara (276, 277) used potentiometric dyes to show that the t tubules have electrical properties similar to the sarcolemma. The t-tubular system represents a large extension of the sarcolemma increasing the cell capacitance, and hence the membrane by a factor of 4–5 (162). Propagation of AP in these tubules might therefore contribute considerably to the ion fluxes associated with excitation, and given the diffusional limitations in these narrow structures, ion perturbations that affect excitability might well occur. Several authors have provided indirect evidence that fatigue may result from inexcitability of the t tubules (26, 27, 79, 163, 387). However, direct evidence has yet to be presented.

The t tubules are less developed in heart muscle than in skeletal muscle, and only ~30% of the sarcolemma is located to the tubular system in ventricular cells (514). A recent study using two-photon confocal microscopy questions earlier data obtained by electron microscopy and claims that the t-tubular system (which they propose to rename sarcolemmal Z rete) is much more extensive (608). Also in the heart the site of coupling between excitation and [Ca$^{2+}$]$_{c}$ release resides in these tubular structures. Again ion perturbations with normal heart activity are small, and failure of t-tubular function probably does not occur (see sect. VI).

The situation may be very different in skeletal muscle. For the t tubules to propagate AP they must be equipped with the membrane proteins that provide excitability and proteins that restore the ion homeostasis. Early investigations in frog muscle clearly showed that diffusion of ions into the t-tubular system was quite slow, with a time constant in the order of several seconds (reviewed in Ref. 14). Almers and Stirling (15) reviewed some of the literature and concluded that compared with surface sarcolemma the density of Na$^{+}$ channels was slightly less in the t tubules; IRK density and Cl$^{-}$ channel density were about the same, whereas the delayed K$^{+}$ rectifier and the Na$^{+}$-K$^{+}$ pump were only present in small amounts in the t tubules. It is also important that similar channel density in the t tubules and the sarcolemma means that four to five times more of the protein is actually located to the tubules due to their size. More recent data actually indicate that the density of Na$^{+}$ channels is high in the t tubules (477). Furthermore, Cl$^{-}$ channel density is probably four to five times higher in the t tubules than in the surface sarcolemma (118, 160), which means that most of the Cl$^{-}$ conductance of the cells resides in the tubules. With regard to distribution of the...
Na\(^+\)-K\(^+\) pump, it is unclear whether the data of Venosa and Horowicz (654) from the frog can be extrapolated to mammalian muscle; possibly the density of Na\(^+\)-K\(^+\) pumps is higher. At least in the mammalian heart the density of Na\(^+\)-K\(^+\) pumps is high in the t-tubular system (457).

The high Cl\(^-\) conductance of the tubules might be quite important in protecting the tubules from becoming inexcitable. The high Cl\(^-\) conductance would reduce release of K\(^+\) and stabilize the E\(_{m}\) (14, 118, 160). Also, the Na\(^+\)-K\(^+\) pump would contribute to maintain the E\(_{m}\), although given the large conductance of the tubules this effect would be small. However, the pumps might be instrumental in clearing accumulated K\(^+\) from the tubule lumen.

The role of K\(^+\) accumulation in the tubules has been elegantly examined in a recent simulation study (664). More than 80\% of the inward rectification of the skeletal muscle cell membrane is located to the t tubules (22), and the consequences of this fact have not been appreciated. In the simulation study the authors conclude that due to the high Cl\(^-\) conductance K\(^+\) accumulation in the t tubules will cause the E\(_{k}\) to become positive relative to E\(_{m}\). Thus an inward K\(^+\) current through the IRK channels plays a major role in clearing K\(^+\) from the t tubules. The authors have not adequately dealt with the role of the Na\(^+\)-K\(^+\) pump, since they have not included an increased pump activity. Because of the stimulation of the pump it will have a strong hyperpolarizing power (see Fig. 4) that will also augment the uptake of K\(^+\) through the IRK channels (see sect. IV A4).

Fitts (201), in his review on muscle fatigue mechanisms, points out that the [Ca\(^{2+}\)] in the t-tubular lumen may also be very important for the function of the voltage sensor. However, it is not known whether tubular [Ca\(^{2+}\)] increases or decreases during muscle activation. Cairns et al. (82) recently provided evidence consistent with Ca\(^{2+}\) depletion in the t tubules might be an important fatigue factor, whereas Bianchi et al. (50) pointed out that increased [Ca\(^{2+}\)] in the t tubules could also be a fatigue factor.

Until direct information can be produced about these fundamental properties of the t-tubular system, their role in fatigue relates to ion perturbations across the tubular membrane will remain unclear. However, there is some indirect evidence for propagation failure in the t tubules. Both appearance of wavy fibrils in the muscle fiber center and a central core of low [Ca\(^{2+}\)] during high-frequency simulation is considered indicative of t-tubular conduction failure (163, 227, 306, 307, 690). It remains an intriguing possibility that conduction failure can occur in the t tubules during high-, moderate- , and low-intensity exercise. For further discussion see section IX.

V. LONG-TERM REGULATION OF POTASSIUM CONTENT AND SODIUM-POTASSIUM PUMP CONCENTRATION IN HEART AND SKELETAL MUSCLE

Despite the redundancy of mechanisms for regulation of K\(^+\) homeostasis as described above, K\(^+\) (content) and/or [K\(^+\)]\(_c\) (concentration) of heart and especially of skeletal muscle can change under certain conditions. This may occur as a consequence of changes in the Na\(^+\)-K\(^+\) pump capacity, but can also occur independently of pump capacity. The conditions that will be elucidated are age-related changes as well as changes related to prolonged sustained alterations in the activity of skeletal muscle, ranging from chronic stimulation through voluntary training activities as well as denervation. The two variables age and activity level may well be interrelated, e.g., the activity pattern changes with age. Furthermore, both K\(^+\) homeostasis and Na\(^+\)-K\(^+\) pump capacity are influenced by the composition of the diet and/or hormonal changes.

A. Age-Related Changes

During a life span, tremendous morphological and functional changes occur for every skeletal muscle, and the degree of maturation at birth is highly variable among species. Data on prenatal Na\(^+\)-K\(^+\) pump concentrations are scarce, but recently it has been shown in pigs that there is a steady but gradual increase of pump content in skeletal as well as heart muscle during the second half of gestation (128). The K\(^+\) conductance increases early in life with differentiation from myoblasts to myotubes (695), although it also increases again later in life with advanced aging together with a simultaneous large reduction in Cl\(^-\) conductance (141, 142). The t tubules that account for up to 80\% of K\(^+\) conductance develop early, causing an increase in membrane surface and cell capacitance (210). Later on, muscle fiber cross-sectional area increases so that muscle fiber membrane area per unit volume muscle tissue decreases. This implies that the total surface area for K\(^+\) release per kilogram muscle mass is highly variable, and precautions must be taken when comparing data related to kilogram wet muscle mass. At least three different postnatal stages have been considered: immature, adult, and old age.

Ouabain binding sites related to wet muscle weight increase dramatically in rat skeletal muscle from birth by up to fivefold over a 4-wk period, where the highest value of ~800 mmol/kg wet wt is attained, and then decline to the level seen in 1-wk-old rats over a period of another 8 wk (Fig. 7) (109, 265, 351, 352). The changes seen in guinea pigs were somewhat different, which is probably due to the fact that these mammals are more mature at birth, and only later do they show the decline seen in rats.
In line with this, the Na\(^{+}\)-K\(^{+}\) pump-dependent energy turnover was around 14% lower for muscle from 7-mo-old compared with 10- to 21-day-old dairy calves (241). Interestingly, when related to the whole muscle, the total number of \(^{3}H\)ouabain binding sites in one whole soleus muscle remained almost constant after the first 4 wk in rat (351). As a matter of fact, this coincides with findings in humans where no change was demonstrated in Na\(^{+}\)-K\(^{+}\) pump concentration from 18 to 80 yr of age since there is no muscle fiber hypertrophy in this period (505), although the same level of pump concentration is reported in children (0–8 yr) as in adults, which is most probably due to the maturity at birth in humans (349). In one study it was observed that the initial increase in \(^{3}H\)ouabain binding sites did not match ouabain-suppressible uptake of K\(^{+}\), which in fact decreased substantially over the same period (352). The reason for this was unclear. It could neither be ascribed to changes of [Na\(^{+}\)]\(_{c}\) nor water content. Changes in body fluid volumes with age are too small to account for any changes in K\(^{+}\) homeostasis (402). In the heart there are only small, if any, age-related changes of Na\(^{+}\)-K\(^{+}\) pump density once adulthood has been reached (389, 560).

In general, in rat muscles, the number of Na\(^{+}\)-K\(^{+}\) pumps is slightly higher in fast-twitch compared with slow-twitch muscle (109, 183, 190). This could correspond to the higher K\(^{+}\)\(_{c}\) and lower Na\(^{+}\)\(_{c}\) in fast-twitch muscles compared with slow-twitch muscles (84, 95, 413). However, species differences do exist. In the pig (264) and in the mouse (113), slow-twitch fibers contain more Na\(^{+}\)-K\(^{+}\) pumps than fast-twitch fibers, whereas K\(^{+}\)\(_{c}\) is still lowest in the slow-twitch fibers (264). Madsen et al. (439) were unable to find any correlation between fiber type composition and number of ouabain binding sites in human muscle, which is in concordance with no significant differences in K\(^{+}\)\(_{c}\) and Na\(^{+}\)\(_{c}\) of human slow- and fast-twitch muscles (589). This explains why no decrease in \(^{3}H\)ouabain binding sites may have occurred (505) despite the age-related selective loss of fast-twitch muscle in humans (390, 403). Functionally, it has been suggested that the smaller M wave during muscle activation in elderly humans can be explained by decreased Na\(^{+}\)-K\(^{+}\) pump concentration (126), which, however, is not supported by the above data on human skeletal muscle. Alternatively, the smaller M wave may be explained by the reduced \(\beta_{2}\)-adrenoceptor-stimulated K\(^{+}\) uptake and hence less efficient stimulation of the Na\(^{+}\)-K\(^{+}\) pump in aged humans (205).

To summarize, therefore, the changes that occur in the density of Na\(^{+}\)-K\(^{+}\) pumps in skeletal muscle in the neonatal period and early parts of life are qualitatively different from those later in life. The abrupt increase that occurs early in some species has been ascribed to an increase of the membrane area and insertion of new proteins in the membrane. If muscle fibers continue to enlarge after this period, as in rats, the number of pumps related to muscle weight and also to intracellular fluid volume decreases. This is due to the reduction of the membrane surface-to-volume ratio, which is an important independent determinant of muscle Na\(^{+}\)-K\(^{+}\) pump density (264). Hence, control of [Na\(^{+}\)]\(_{c}\) and [K\(^{+}\)]\(_{c}\) will be less efficient in the sense that the same number of pumps controls a larger amount of intracellular ions. However, this does not seem to occur in humans. Finally, it is of note that the Na\(^{+}\)-K\(^{+}\) pump concentration in the heart is little affected by age and that even at old age the Na\(^{+}\)-K\(^{+}\) pump concentration in skeletal muscle may be increased by physical activity (362). This implies that a possible age-related decrease in pump concentration may be due to a decreased physical activity level. A functional consequence may be the increased muscle excitability (M wave) and twitch potentiation found in elderly people after training (287, 288).

**B. Activity-Induced Changes**

Muscle activity patterns have a profound effect on the long-term regulation of the concentration of Na\(^{+}\)-K\(^{+}\) pumps in skeletal muscle, but interestingly less so on the K\(^{+}\) content. A large number of studies have manipulated the muscle activity by either increasing or decreasing the contractile activity and monitoring pump concentration and/or K\(^{+}\) content in various muscles from different species. This ranges from various voluntary activation patterns such as running, swimming, and cycling to electrical stimulation (185, 187, 238, 239, 353, 354, 362, 364, 401, 439, 463, 618, 620). One of the earliest studies, conducted on rats, demonstrated a 5% increase in thigh muscle K\(^{+}\) content after 10 wk of swim training, although no change was recorded in the heart muscle (499). In contrast, 35
days of chronic stimulation in the rabbit decreased K\(^+\) content by 53\% (618). The largest effect on the Na\(^+\)-K\(^+\) pump (165\% increase in concentration) was reported in the dog gracilis muscle after 6 wk of run training (364). In a number of other studies, decreased physical activity was attained by detraining, bed rest, immobilization, suspension, or denervation and reinnervation (109, 111, 113, 354, 401, 440, 483). The results of some of the studies are compiled in Table 5. The evidence convincingly suggests that increased muscle activity increases Na\(^+\)-K\(^+\) pump concentrations. There was only one study in which no significant increase was found, which is probably due to the lack of specificity regarding the involvement in the general conditioning program of the muscle biopsied for Na\(^+\)-K\(^+\) pump analysis (353). The magnitude of changes depends on the change in intensity and the duration, generally in the range of 10–40\%, although as mentioned above, an increase up to 165\% was reported. Interestingly, Green et al. (237) recently reported downregulation by almost 14\% of the Na\(^+\)-K\(^+\) pump during training in normobaric hypoxia, which raises intriguing questions as to the specific stimulus for variation of the muscle content of Na\(^+\)-K\(^+\) pumps. Some conflicting data are found regarding chronic stimulation, which could be regarded as an endurance form of training. At a stimulation frequency of 10 Hz of the EDL muscle in rabbits, a significant increase of Na\(^+\)-K\(^+\) pump density had already occurred after 4 days, and by the end of the stimulation period of 50 days (end point of study), it had increased further so that Na\(^+\)-K\(^+\) pump concentration was 86\% above contralateral

<table>
<thead>
<tr>
<th>Muscle Activity (Mode and Duration)</th>
<th>Muscle K(^+) Content</th>
<th>Muscle Na(^+)-K(^+) Pump Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control, mmol/ kg wet wt</td>
<td>Change, % of control</td>
<td>Control, (\mu)mol/ kg wet wt</td>
</tr>
<tr>
<td>Human, elderly vastus lateralis</td>
<td>Swimming, running, strength training 12–17 yr</td>
<td>Not measured</td>
</tr>
<tr>
<td>Human vastus lateralis</td>
<td>Endurance running 6 wk</td>
<td>Not measured</td>
</tr>
<tr>
<td>Human vastus lateralis</td>
<td>Endurance, cycling 6 days</td>
<td>Not measured</td>
</tr>
<tr>
<td>Human vastus lateralis</td>
<td>Endurance, skiing 5 mo</td>
<td>Not measured</td>
</tr>
<tr>
<td>Human vastus lateralis</td>
<td>Military training 10 wk</td>
<td>Not measured</td>
</tr>
<tr>
<td>Human vastus lateralis</td>
<td>Sprint, cycling 7 wk</td>
<td>Not measured</td>
</tr>
<tr>
<td>Rat EDL, gastrocnemius, soleus</td>
<td>Swimming 6 wk</td>
<td>Data not presented</td>
</tr>
<tr>
<td>Guinea pig gastrocnemius, soleus</td>
<td>Running 3 wk</td>
<td>107 (Ga)</td>
</tr>
<tr>
<td>Rabbits EDL</td>
<td>Chronic 10-Hz stimulation 1, 2, 3, 4, 10, 50 days</td>
<td>Not measured</td>
</tr>
<tr>
<td>Rat EDL, tibialis anterior, soleus</td>
<td>Chronic 10-Hz stimulation 1 day (~7 days)</td>
<td>104 (EDL)</td>
</tr>
<tr>
<td>Human vastus lateralis</td>
<td>Detraining 4 wk</td>
<td>107 (Ta)</td>
</tr>
<tr>
<td>Rat soleus</td>
<td>Immobility 1 wk</td>
<td>90 (Sol)</td>
</tr>
<tr>
<td>Guinea pig gastrocnemius</td>
<td>Immobility 3 wk</td>
<td>150 (EDL)</td>
</tr>
<tr>
<td>Rat EDL, soleus</td>
<td>Suspension</td>
<td>130 (Sol)</td>
</tr>
<tr>
<td>Rat in vivo EDL, soleus</td>
<td>Denervation 1 wk</td>
<td>Data not presented</td>
</tr>
<tr>
<td>Rat, in vitro EDL, gastrocnemius, soleus</td>
<td>Denervation 1 wk</td>
<td>115 (EDL)</td>
</tr>
</tbody>
</table>
| EDL, extensor digitorum longus; Ga, gastrocnemius; Sol, soleus; Ta, tibialis anterior.
unstimulated control muscles (238). In contrast, 10-Hz stimulation for 1 and 7 days in various rat hindlimb muscles did not cause significant changes of the Na\(^+\)-K\(^+\) pump concentration (185). The reason for this discrepancy may be put down to species differences, including differences in caged activity level between rat and rabbit as well as the masking by the simultaneous age-related changes. Finally, shivering induced by cold exposure also increases the Na\(^+\)-K\(^+\) pump density of skeletal muscles, which is probably due to the increased activity of the muscles (264).

Inactivity of a muscle will induce a decrease in the Na\(^+\)-K\(^+\) pump concentration as demonstrated in animal studies with extreme immobilization (109, 320, 354, 401), while the changes in muscle K\(^+\) are less consistent. For instance, suspension induced a loss of K\(_{\text{c}}\) and accumulation in Na\(_{\text{c}}\) in rat soleus and diaphragm but not in EDL muscles, and a subsequent denervation of the soleus muscle caused a recovery of its electrolyte contents (483). It is well known that immobilization causes atrophy, i.e., smaller fiber diameter which, interestingly, per se would cause only minor changes in electrolyte contents but large increases in concentration of Na\(^+\)-K\(^+\) pumps. For instance, in sheep, when one of their hindlimbs was immobilized in a plaster splint for 9 wk, a reduction in muscle volume of 15% was estimated from the reduction in muscle fiber circumferences, which corresponded to an 18% increase in surface membrane-to-volume ratio. Nonetheless, Na\(^+\)-K\(^+\) pump concentration per tissue volume was decreased by 39%, indicating a tremendous reduction in pump density related to membrane surface area with inactivity (320). In one study on detraining in humans for 4 wk, no significant decrease was seen, which may be due to slow downregulation when normal daily life activity is still maintained (440). In line with this, workers suffering from work-related musculoskeletal disorders maintained normal Na\(^+\)-K\(^+\) pump concentrations despite the fact that painful muscles forced them to be largely inactive and that they lived on workers compensation pension (596).

The mechanism by which exercise training increases Na\(^+\)-K\(^+\) pump content was recently investigated by Tsakiridis et al. (643). They found with immunoblotting technique that 1 h of exercise increased the amount of \(\alpha_1\) and \(\alpha_2\)-subunit protein in plasma membranes from both fast- and slow-twitch fibers in rats. However, the subcellular source of this protein was unclear. They found an enhanced mRNA signal only for \(\alpha_1\) in slow-twitch muscle and only for \(\beta_2\) in fast-twitch muscle and dismissed the idea that the increase in pump protein in membrane fractions is the result of de novo synthesis, since they did not find increased protein levels in the crude homogenate. Their conclusion is that pump proteins are obtained from an intracellular pool or that membrane protein degradation is abruptly decreased. However, they did not observe reciprocal changes of the pump proteins in any of the other subcellular fractions.

All in all, the results indicate that increased muscle activity induces an increased capacity to clear K\(^+\) from the interstitial space as confirmed by a number of studies (207, 344, 442, 454, 462, 463, 468, 637) and that this increased capacity may last for some time even after return to normal muscle activity level. The physiological significance of this adaptation has recently been reviewed by McKenna (461), with emphasis on regulation in humans. The review points out that increased muscle Na\(^+\)-K\(^+\) pump content may not directly account for the improved K\(^+\) regulation and exercise performance after training. Instead, increased efficiency of pump activation may be important. This matter is further discussed in section VIII.C.

It has been speculated that increased Na\(^+\)-K\(^+\) pump capacity subsequent to a training period may also affect K\(_{\text{c}}\) in the skeletal muscles at rest. Small changes have been reported, e.g., Nöcker et al. (499) found a 5% increase in skeletal muscle at rest and Knochel et al. (364) reported a 6% increase in [K\(^-\)]\(_{\text{c}}\), together with a membrane hyperpolarization. In contrast, with chronic stimulation, which may well induce increases in Na\(^+\)-K\(^+\) pump concentration, the changes in muscle tissue electrolytes are rather similar to the acute changes reported during exercise, that is, a marked dissipation of cellular K\(^+\) and accumulation of Na\(^+\) (185, 618). Furthermore, inactivity induced a reduction of K\(_{\text{m}}\) as well as a decrease in [K\(^-\)]\(_{\text{c}}\). However, in several studies, no statistically significant changes were found (see Table 5). From a functional point of view, the maintenance of [K\(^-\)]\(_{\text{c}}\) and [K\(^+\)]\(_{\text{c}}\) is important, since the excitation and AP propagation depend on concentration gradients within narrow limits. Interestingly, it has been shown that with restriction of motor activity a parallel decrease was seen in K\(^+\) and water content expressed per kilogram dry weight both in skeletal muscle and heart muscle so that the K\(^+\)/H\(_2\)O or [K\(^+\)]\(_{\text{c}}\) was maintained (719). The effect of training on heart muscle electrolyte content has not been examined by many investigators. Interestingly, Nöcker et al. (499), many years ago now, showed that the heart K\(_{\text{c}}\) was little affected by a training regimen.

C. K\(^+\) Availability

To maintain whole body K\(^+\) homeostasis, diet must consist of a quantity of K\(^+\) matching the K\(^+\) excretion from the body in urine, feces, and sweat, which for adult humans amounts to a minimum of \(-20\) mmol/day (for references, see Ref. 473). The dietary effects on K\(^+\) balance have been reviewed in depth by Clausen and Everts (103). Starvation or K\(^+\)-deficient diet will after some weeks cause K\(^+\) depletion, e.g., a reduced K\(_{\text{c}}\) and [K\(^+\)]\(_{\text{c}}\) in
skeletal muscle and to a minor extent in heart muscle (42, 350, 474, 504, 548). The same condition may be induced by chronic diuretic treatment (156). Furthermore, dietary Mg$^{2+}$ deficit is associated with a reduction in skeletal muscle K$^+$ too (156, 503), although, if the diet is rich in K$^+$, the level of muscle K$^+$ remains normal (96). The mechanism of the interrelationship between muscle Mg$^{2+}$ and K$^+$ remains puzzling, although probably unrelated to any effect of Mg$^{2+}$ on the Na$^+$/K$^+$ pump-mediated K$^+$ uptake in skeletal muscle (151). It is well known clinically that a K$^+$ deficit may not be identified from plasma [K$^+$], since this may be maintained at normal level despite a large decrease of muscle K$^+_c$ (503). Of note in this context is the fact that different reference ranges for [K$^+$]$_{\text{v, mix}}$ have been reported in different populations, e.g., as low as 2.8–4.1 mM in the Barbadian population (231), and it remains to be revealed if this is due to differences in climate or nutrition, or whether different populations may show inherent differences in [K$^+$]$_c$ levels. As yet, we lack the necessary knowledge for the clinical assessment of abnormal [K$^+$]$_c$ levels in aboriginals.

The ability of the organism to handle an extra K$^+$ load is highly dependent on the prevailing training status and the level of K$^+$ intake. Zorbas et al. (720) recently showed that in subjects performing regular physical activity an oral K$^+$ load was excreted much more slowly than in inactive subjects even though the latter group had lost a large amount of K$^+$ during the inactivity period. An intragastric load of K$^+$ was given to normal rats and to rats that had been K$^+$ depleted over a 4-wk period causing ~40% reduction of K$^+_m$. In normal rats, K$^+_m$ (muscle K$^+$ content) increased by 10%, and [K$^+$]$_p$ was unaffected. In contrast, although K$^+_m$ increased by 64% after the intragastric K$^+$ load in the K$^+$-depleted rats, normal muscle K$^+_m$ was not reached and [K$^+$]$_p$ increased to almost 1.5 times above normal resting level (548). This indicates that after severe K$^+$ depletion, the capacity of the muscle to accumulate K$^+$ or to clear K$^+$ from the plasma is impaired. The explanation for this finding may be that in skeletal muscles in situ, K$^+$ depletion leads to a large decrease in Na$^+$-K$^+$ pump concentration (350, 504). Thompson and co-workers (633, 634) recently reported that the reduction of K$^+_m$ during hypokalemia is preceded by a reduced cell content of the α2-isoform of the catalytically active subunit of the Na$^+$-K$^+$ pump. This is in contrast to findings in cultured cells, where K$^+$ loss is associated with an increase in Na$^+$-K$^+$ pump concentration (190). This discrepancy emphasizes the importance of studies on intact animals including humans, because the priority to regulatory adaptations may be totally diverse; in the tissue culture, prime priority is simply to improve K$^+$ uptake during restricted K$^+$ availability, i.e., Na$^+$-K$^+$ pump concentration is increased. However, in the intact organism, highest priority is given to vital organs such as the heart, brain, liver, and erythrocytes that maintain normal K$^+_c$ for weeks (for reference, see Ref. 111). Therefore, the role of the muscle in the K$^+$-deficient condition is to serve as a K$^+$ reservoir, “leaking” K$^+$ to the plasma to supply other tissues. For this purpose, a downregulation of the Na$^+$-K$^+$ pump is a logical consequence, and therefore, the muscle tissue is considered to play a key role in the maintenance of plasma [K$^+$].

An interesting observation on the interplay between muscle K$^+_c$ and Na$^+$-K$^+$ pump density was presented by Akaike (9, 10). Denervation does not seem to cause major changes of [K$^+$]$_c$ in normal rats (394), but when muscles were denervated in K$^+$-deficient rats the muscle K$^+_m$ increased. This was interpreted as a regulation by the central nervous system (9, 10). In a later study, Clausen et al. (111) came to the same conclusion and in addition found that this substantial gain of K$^+_m$ took place in face of an unchanged number of [$^3$H]ouabain binding sites. By comparison with immobilization studies, they convincingly concluded that muscle activity as such regulates muscle K$^+_m$ independently of the innervation, indicating that the denervation-induced increase in K$^+_m$ was simply due to reduced muscle activity.

How muscle K$^+_c$ is actually controlled remains an open question. Under some circumstances there is a close relationship between Na$^+$-K$^+$ pump density and K$^+_m$. However, this relationship may be broken (111, 155, 264, 320, 534). Clearly, both muscle activity and dietary intake have independent effects on both K$^+_m$ and Na$^+$-K$^+$ pump density. Hence, factors other than Na$^+$-K$^+$ pump density also control the K$^+_c$. It is very probable that variable cell membrane permeability to K$^+$ is also an important determinant. Additionally, as far as we know, nobody has examined the importance of fixed negative charges in the cell as an independent determinant of cellular K$^+$ content. CrP is the most abundant intracellular strong anion, and one might expect that changes in cell content of this compound would alter the K$^+_c$ as well. Finally, muscle K$^+_c$ is related positively to glycogen content when expressed per kilogram dry weight (43), whereas hypokalemia is associated with low glycogen stores (363). Because increased muscle glycogen content is also associated with increased water content, the K$^+$ versus glycogen relationship may be due to the regulation of [K$^+$]$_c$ (45).

D. Hormones

Hormones that are involved in the acute regulation of the Na$^+$-K$^+$ pump activity are discussed in sections III and VIII; only long-term regulation is briefly summarized here. Long-term regulation of the Na$^+$-K$^+$ pump density can be achieved by thyroid hormones. This topic has recently been extensively reviewed (181). Hyperthyroid animals may have 10 times higher pump densities in skeletal muscle compared with hypothyroid animals. The effect of
thyroid hormones seems to be more pronounced on slow-
twitch than on fast-twitch muscle fibers (348), and in the
rat diaphragm, the \( \alpha \)-isoform (ouabain sensitive) is
specifically upregulated (247). The mechanism underlying
this effect is probably an initially increased passive permeability to Na\(^+\) and K\(^+\) (103, 182), since an independent
effect of thyroid hormones on muscle Na\(^+\)-K\(^+\) pump density
could not be detected in cold-exposed pigs (264).
More recently, Harrison and Clausen (263) found that
the number of Na\(^+\) channels is also increased at an early
stage in hyperthyroidism, possibly contributing to the
fatigue in hyperthyroid patients.

Kubota and Ingbar (375) examined the relationship
between thyroid status and the acute effect of cate-
cholamines on extrarenal K\(^+\) disposal during a standard-
ized K\(^+\) infusion in rats. They conclude that both thyroid
hormones and \( \beta \)-adrenoceptor agonists increase the rate
of K\(^+\) disposal, and they also found a positive interaction
effect. However, their results are very difficult to interpret
since they did not take into account the significant inhi-
bition of the Na\(^+\)-K\(^+\) pump rate that occur when [K\(^+\)]\(_c\) is
reduced below 4 mM.

When infused as dexamethasone in pharmacological
doses, glucocorticoids induce an upregulation of the
Na\(^+\)-K\(^+\) pump which occurs without any change of muscle
K\(^+\) (155). However, in physiological concentrations,
endogenous corticoids seem to be of minor importance as
indicated from studies on adrenalectomy [206; for review,
see also Dørup (150)]. In some studies aldosterone injec-
tion in rat or guinea pig over a 2- to 3-wk period caused an
upregulation of the Na\(^+\)-K\(^+\) pump in heart muscle but not
in skeletal muscle (271, 621). A small downregulation
accompanied by loss of K\(^+\)_m in response to aldosterone
was also reported in skeletal muscle (155). This lack of
effect in skeletal muscle may be caused by a dual effect of
aldosterone. It is possible that the direct effect on muscle
is counteracted by its hypokalemic effect (155). Evidence
has been presented that this downregulation of Na\(^+\)-K\(^+\)
pumps with K\(^+\) deficiency is a general phenomenon that
is neither species dependent nor dependent on the cause
of the K\(^+\) deficiency state (110, 150). In neonatal heart
cells, thyroid hormones and glucocorticoids have been
reported to differentially regulate the various \( \alpha \)- and
\( \beta \)-subunit isoforms (509).

Finally, chronic treatment of guinea pigs with \( \beta \)-ad-
renoceptor agonists (isoprenaline or terbutaline) reduces
the density of Na\(^+\)-K\(^+\) pumps (172), which may be due to
the simultaneous downregulation of the density of \( \beta \)-ad-
renoceptors and a close regulation between these recep-
tors and Na\(^+\)-K\(^+\) pumps in skeletal and cardiac muscle
(173). However, in humans, the time course and extent of
K\(^+\) perturbations with exercise were similar after acute
compared with "chronic" or rather prolonged (2-wk) \( \beta \)-ad-
renoceptor blockade (245).

This brief account of hormonal effects on K\(^+\)_m and
Na\(^+\)-K\(^+\) pump density is only intended to show that hor-
monal effects can be marked. However, within a physi-
ological range, hormonal effects on Na\(^+\)-K\(^+\) pump density
are small, and quite often, other concomitant changes like
dietary intake and muscle activity are the more important
stimuli. We can therefore conclude that the signaling
pathways for changes of K\(^+\)_m and Na\(^+\)-K\(^+\) pump density
are multiple, the two are not always correlated, and today
we do not know all these pathways or their interrela-
tionship.

VI. POTASSIUM SHIFTS OF THE
WORKING HEART

K\(^+\) balance in the heart has been the focus of much
research of which a large part is related to K\(^+\) shifts
during myocardial ischemia and will not be extensively
reviewed here. However, K\(^+\) balance in the normal beat-
ing heart has several features that are in contrast to the
test of K\(^+\) balance in skeletal muscle, and which may
be helpful in understanding the basic mechanisms for
control of K\(^+\)_m. First, anatomy of cardiac muscle is differ-
ent since cells are arranged in bundles surrounded by a
sheath and they are adjoined through the intercalated
disk. Thus there is a subendothelial space, which at least
in the isolated preparations can accumulate considerable
amounts of K\(^+\). Also, the membrane area of the disk
region is quite large and has been estimated to constitute
90% of the total cell membrane (515). The volume of the
intercellular space in the disk region is small. This struc-
ture creates intercellular compartments in which K\(^+\)
may accumulate (116). Interestingly, the density of Kv1.5 pro-
teins that form part of voltage-sensitive channels are
much more abundant in the disk region compared with
the surface membrane (447). The t tubules are less exten-
sive in the heart than in skeletal muscle (see sect. nD),
although the disk region may have a similar function with
regard to control of E_m. Second, as described in section iii,
the concentration of Na\(^+\)-K\(^+\) pumps is considerably
higher than in skeletal muscle which, when taken to-
gether with the low frequency of the heart beat, allows for
a much tighter control of [Na\(^+\)]\(_c\) and [K\(^+\)]\(_c\).

The introduction of ion-selective microelectrodes
made it possible to record [K\(^+\)]\(_m\) during single AP in a
number of isolated cardiac preparations (359–361, 378).
In frog ventricular muscle depending on recording depth,
[K\(^+\)]\(_m\) increased by 0.5–1.5 mM during most of the AP,
which lasts 500–800 ms (361). This means that K\(^+\) was
released from the cells both during the plateau phase and
during the rapid repolarization and that length of the AP
in part determined the total release, even though mem-
bane conductance is actually quite low toward the end of
the plateau phase (677). Interestingly, and in contrast to
skeletal muscle, as much as 80% of the total K\(^+\) release is
independent of the beating frequency, indicating that a large fraction of the release and uptake of K$^+$ in heart muscle occurs during diastole (177).

The amount of K$^+$ release associated with a single AP has been difficult to quantify also in the heart. Ion-selective electrodes in the extracellular space provide some information, but quantitative data rely on estimates of diffusion rates and size of extracellular space. Kline and Morad (361) calculated a net release of 10 pmol/cm$^2$ per AP. By using ion-selective electrodes in the coronary sinus, Ellingsen et al. (177) were able to quantify release by a slightly different approach compared with the technique described by Hallén and Sejersted (254). They calculated K$^+$ loss rate that after an initial peak fell mono-exponentially. By extrapolation they found a release of 4 nmol·g$^{-1}$·beat$^{-1}$ which given that membrane surface is ~3,000 cm$^2$/g is an order of magnitude lower than the above estimate. Also Kline and Morad (361) noted that their estimate was an order of magnitude greater than required to discharge the membrane capacitance. The reason for this discrepancy is unclear, but the lower estimate fits better with observations on Na$^+$/K$^+$ pump rates (176).

Following an increment in heart rate the K$^+$ that accumulates in the interstitium with each AP will not be completely taken up again, and [K$^+$]s will gradually rise as K$^+$ dissipates from the myocardial cells (249, 385). The K$^+$ accumulation in the interstitial space is transient, since net release stops after Na$^+$/K$^+$ pump activation, and [K$^+$]s will go back to normal as the initially released K$^+$ diffuses into the bath (360, 361, 378, 676) or is rapidly washed out with the perfusing blood (177, 314). Following a reduction in heart rate, [K$^+$]s was transiently below normal resting level (360, 378), thus indicating a continued uptake of K$^+$ when release was suddenly reduced.

As reviewed by Morad (478) and by Cohen and Kline (116), the extracellular accumulation of K$^+$ with increased beating frequency will depolarize the cells and cause a shortening of the AP. This effect is achieved through an effect on the IRK channel. It was also pointed out that the depolarizing effect of extracellular K$^+$ accumulation may be offset by activation of the Na$^+$/K$^+$ pump. Because the released K$^+$ is so rapidly washed away in vivo, the physiological relevance of the observed extracellular K$^+$ accumulation in isolated tissue specimens is questionable (116).

Originally Hajdu (249) suggested that the K$^+$ loss associated with increased beating frequency of the heart could explain the staircase phenomenon, namely, that contractility is increased. Because there is a reciprocal relationship between [K$^+$]s and [Na$^+$]c (62, 115), the Na$^+$/Ca$^{2+}$ exchange rate will go down and the cells will gain Ca$^{2+}$. This is still a very likely explanation for the force-frequency relationship (171, 266), although recently it was shown that the rate at which Ca$^{2+}$ can be sequestered by the sarcoplasmic reticulum is also important (472). In contrast, the rise in tension in skeletal muscle with increasing stimulation frequencies is not in the same way causally related to the rise in [Na$^+$]c. Skeletal muscle expresses an mRNA signal for the Na$^+$/Ca$^{2+}$ exchanger (524), and Na$^+$/Ca$^{2+}$ exchange has been identified in frog, rabbit, and mouse skeletal muscle (25, 149, 232, 308). However, the skeletal muscle Na$^+$/Ca$^{2+}$ exchanger has a low capacity and low affinity for intracellular Ca$^{2+}$ and is therefore probably only important in its normal forward Ca$^{2+}$-expelling mode during tetani and other conditions that cause sustained elevation of [Ca$^{2+}$]c (25). These data therefore fit with a lack of relationship between a rise in [Na$^+$]c, and force of contraction in skeletal muscle.

Experiments with $^{86}$K and with continuous recording of [K$^+$] in the vein of the perfused intact heart have confirmed that following an increment in heart rate a bolus of K$^+$ is lost from the myocardium and that the same amount is reclaimed when beating frequency is reduced (177, 233, 314, 385). Figure 8 shows a typical tracing of [K$^+$]c during and after a sudden increment in beating frequency by 50 beats/min of the pig heart (177). Compensation of the increased release is complete within 1–2 min in accordance with the prediction of the model presented in section III. The absolute changes in [K$^+$]s are quite small, well below 0.2 mM corresponding to <0.2% of the tissue K$^+$ content (314). Clearly, the effects of transiently raised [K$^+$]s will only affect cardiac function and not other organs, since the loss is so small, and only for a brief period, since [K$^+$]s is rapidly normalized by washout in a well-perfused myocardium.

![Figure 8. Coronary sinus plasma K$^+$ concentration ([K$^+$]s) measured by an ion-selective minielectrode during and after a period of cardiac pacing at a rate of 50 beats/min above resting level. Experiments were performed on anesthetized pigs. Also shown is the plasma [K$^+$] in the left atrium ([K$^+$]a). From Ellingsen et al. (177), with permission from Elsevier Science.](https://physrev.physiology.org/DownloadedFrom)
Quantitative information about K\(^+\) uptake rates have been obtained by using ouabain. Ellingsen et al. (176) infused \(^{3}H\)ouabain as a bolus into the coronary artery of intact pig hearts and measured the ensuing transient K\(^+\) loss. By comparing the loss with the amount of bound \(^{3}H\)ouabain, they concluded that in control conditions the Na\(^+\)-K\(^+\) pump worked at 16\% of its maximum capacity. A similar conclusion was reached by Herzig et al. (281).

Interestingly, catecholamines have a profound effect on K\(^+\) shifts in the beating heart. Exchange rate of \(^{42}K\) is doubled (16), release of K\(^+\) with each AP is doubled, and almost 50\% of the maximum Na\(^+\)-K\(^+\) pump rate is exploited (177). Sudden infusion of catecholamines leads to a substantial uptake of K\(^+\) by the heart muscle (566). The effect seems to be mediated by \(\beta_{1}\)-adrenoceptors (175) and can be seen after stimulation of cardiac sympathetic nerves (179). Also, activation of \(\alpha\)-adrenoceptors causes a small gain of K\(^+\) by cardiac muscle (178). The effect of catecholamines on cardiac K\(^+\) shifts led the authors to propose that these hormones seem to increase the sensitivity of the Na\(^+\)-K\(^+\) pump to intracellular Na\(^-\) (175, 176).

During ischemia, cellular K\(^+\) is rapidly dissipated and accumulates in the interstitial space where [K\(^-\)]\(_{a}\) may reach between 10 and 15 mM in the course of few minutes (289, 293, 517, 680, 694, 712). The rate of rise of [K\(^+\)]\(_{a}\) is to some extent dependent on heart rate (532, 676, 684), and at \(\sim 11.5\) mM mechanical activity ceases (1). It is in this context interesting to note that during exercise the heart may be exposed to a [K\(^+\)]\(_{a}\) of almost 9 mM as discussed in section VIII. After this initial rise of [K\(^+\)]\(_{a}\) during ischemia follows a period of 10–20 min where [K\(^+\)]\(_{a}\) remains stable between 10 and 15 mM before it rises further and can reach concentrations well above 20 mM (289, 680). This latter phase of rise of [K\(^+\)]\(_{a}\) reflects irreversible injury of the cardiomyocytes. The mechanism of the ischemia-induced dissipation of cellular K\(^+\) has been much debated. Aksnes (11) and Wilde and Aksnes (697) proposed that opening of K\(^+\) channels was not very important, although a massive opening of K\(_{ATP}\) channels probably occurs (366). Increased membrane conductance to K\(^+\) will not lead to dissipation of cellular K\(^+\) by itself. It is only provided that a depolarizing inward current has been established, that an outward K\(^+\) current will in turn also be established. They also pointed out that, given the high conductance to K\(^+\), the required depolarization was very small. This hypothesis has recently been corroborated (581).

The consequences of a high [K\(^+\)]\(_{a}\) on cardiac function are first of all related to the effect on membrane potential and excitability. Not only during local ischemia, but also during high-intensity exercise the heart cells will be exposed to significantly elevated [K\(^+\)]\(_{a}\). At a [K\(^+\)]\(_{a}\) in the range of 8–9 mM, the AP in the heart is shortened (678, 684). Paterson et al. (519) observed reduced AP amplitude, reduced upstroke velocity, and shorter AP. This is because the cells become depolarized and have an increased conductance to K\(^+\) due to the effect of [K\(^+\)]\(_{a}\) on IKr. Especially the increased background conductance will contribute to decreased rate of rise of the AP and slowed conductance (683). These changes are considered highly arrhythmogenic. Notably, some of these effects disappeared when the cells were exposed to high extracellular Ca\(^{2+}\) and catecholamines (519), and also mechanical performance was restored (399, 400, 507, 508, 518). Thus, during exercise with a normal activation of the sympathoadrenergic system, there is an interaction between K\(^+\), catecholamines, and H\(^+\) that seems to protect against the harmful effects that each of these factors can have on the heart.

We conclude that in the heart the K\(^+\) balance is normally very tightly controlled, and the K\(^+\) shifts associated with alterations in beating frequency and catecholamine stimulation are moderate. At the same time, however, local accumulation of K\(^+\) during ischemia does significantly affect myocardial function.

VII. POTASSIUM IN ACID-BASE BALANCE

Profound shifts of both K\(^+\) and lactate occur during exercise at high muscle power, and extracellular [K\(^+\)] and [H\(^+\)] increase almost in parallel, seemingly indicating a tight causal relationship (332, 466, 467, 554, 570, 595). However, the same studies also showed that at cessation of exercise the muscle rapidly regains its K\(_{in}\) whereas lactate and H\(^+\) continue to move out of the cell. Thus there is no mandatory coupling between fluxes of these ions across the muscle cell membrane. In the recovery situation K\(^+\) is in fact taken up by a cell that may still have a very low pH. Hence, intracellular acidosis can be ruled out as a direct cause of loss of K\(_{in}\) during exercise. In fact, as described below, alterations of [K\(^+\)] in any one compartment is a determinant of pH, not vice versa.

As described by Stewart (623), pH both in the extra- and intracellular compartments is determined by the concentration of other ions, on the one hand strong anions and cations (strong because they are completely dissociated), and on the other hand proton buffers of which the HCO\(_{3}\) system is the most important. pH and HCO\(_{3}\) are the dependent variables. The independent variables comprise the difference between concentrations of strong anions and cations, the so-called strong ion difference (SID), PaCO\(_{2}\), and the sum of weak acids and bases (non-HCO\(_{3}\) buffers). If these independent variables are known as well as the dissociation constants of the buffers, pH can then be calculated. Thus, by influencing SID, changes in [K\(^-\)]\(_{a}\) or [K\(^+\)]\(_{a}\) will contribute to control of pH even in the absence of any transport coupled to H\(^+\).

In a comprehensive series of publications, Lindinger...
and co-workers (409, 412, 414–418, 421), Heigenhauser and co-workers (273, 274), and Kowalchuk et al. (372) investigated the ion shifts that occur with high-intensity exercise and muscle activity both in rats and humans, and they described the effect of changes on pH in terms of the mathematical coupling that Stewart (623) put forward. The independent variables in $V_{c\text{r}}$ that are altered especially during high-intensity exercise comprise first of all $K^{+}$, CrP, lactate, and $P_{\text{CO}_{2}}$. A fall in $[K^{+}]_{v}$ and a rise of the intracellular lactate concentration will reduce SID and has been claimed to explain >90% of the intracellular $H^{+}$ accumulation (372, 407). As pointed out in section IV.B.2, the concomitant swelling of the cells will amplify the reduction of $[K^{+}]_{v}$, and will also by itself reduce SID. A fall in CrP which usually occurs quite early during exercise will have an alkalinizing effect since it has a $pK_{a}$ of 4.5 and can be regarded as a strong ion (412). On the other hand, $P_{\text{CO}_{2}}$ in venous blood will rise and thus maintain $HCO_{3}^{-}$ in the muscle and therefore contribute somewhat to the acidosis.

Also extracellularly SID is reduced during high-intensity exercise, contributing by as much as 80% to the lowering of pH (417). However, in contrast to the situation in $V_{c\text{r}}$, $K^{+}$ contributes relatively little to the change in extracellular SID, and it is mostly caused by other strong ions.

This physicochemical approach to understanding the relationship between pH and other ions is quite useful for getting a quantitative impression. However, this approach cannot be used to clarify ion transport mechanisms across the sarcolemma as discussed by Sejersted (565). Above all, it is quite clear that the cell is equipped with transport proteins that carry $H^{+}$ or $OH^{-}$. If such transport occurs without accompanying ions, a large membrane potential can be created as in the mitochondria. However, most often electrical neutrality is maintained, meaning that a strong ion is also moved across the membrane so that the relationship between SID and pH is still valid.

Recently, Wasserman et al. (671) claimed that the loss of $K^{+}$ from an exercising muscle could be attributed to the intracellular decrease of CrP. Because CrP is negatively charged, the authors claimed that the Donnan equilibrium would change so that $K^{+}$ would be released. Their argument was based on measurements of $[HCO_{3}^{-}]$ in the femoral vein, whereby the authors observed that $[K^{+}]_{c}$ and $[HCO_{3}^{-}]_{c}$ both increased initially, indicating that $H^{+}$ was taken up in exchange for $K^{+}$. There are, however, several factors that complicate this interpretation. First, at the onset of exercise, the muscle takes up fluid, and the rise of hematocrit in the femoral vein closely follows the rise in $[K^{+}]_{v}$ albeit to a relatively lesser degree (246). Therefore, the 1 mM rise in $[HCO_{3}^{-}]$ that Wasserman et al. (671) observed could be fully explained by the hemoconcentration and therefore does not satisfactorily account for an uptake of $H^{+}$ by the muscle cells. Second, as outlined in section IV, a Donnan equilibrium is based on the high permeability of the cell membrane to both the cation and the anion. CrP does not pass the sarcolemma and therefore will not affect the electrochemical driving force for $K^{+}$. Also, there is no identified molecular mechanism by which $H^{+}$ can enter the cell in exchange for $K^{+}$. A change of CrP will affect the dependent variables, and not the other strong ions as outlined above.

We therefore conclude that $K^{+}$ contributes to pH regulation especially in the intracellular compartment by its effect on SID. However, pH changes and changes in intracellular CrP cannot substantially affect transmembrane release and uptake of $K^{+}$ which is primarily related to electrical events and the pumping of the $Na^{+}$-$K^{+}$ pump.

**VIII. POTASSIUM SHIFTS WITH SKELETAL MUSCLE ACTIVITY**

The loss of $K^{+}$ from a working muscle has in theory been linked to several different processes. The primary event is an increase in release that is either transiently or more permanently not matched by an increased uptake. As outlined above in skeletal muscle and in heart muscle it seems that the release of $K^{+}$ from the cells is not causally linked to volume changes or intracellular acidosis. There is compelling evidence that the repolarizing $K^{+}$ current is the single most important cause for this $K^{+}$ release. However, there is little consensus on the magnitude of these repolarizing currents during the AP.

A large variety of experimental models have been used to quantify $K^{+}$ shifts between tissue compartments with skeletal muscle activity. The models disclose different steps in the chain of events beginning with the unidirectional release from the intracellular or cytosolic compartment, $V_{c\text{r}}$, to the surface of the cell membrane which corresponds to the interstitial space, $V_{s}$, in intact muscle. The intracellular compartment in muscle contains the largest total amount of $K^{+}$ by virtue of its large volume and high $[K^{+}]$. However, the largest relative changes in $[K^{+}]$ necessarily occur inside the t tubules and in the interstitial space simply because of their smaller size and due to the fact that $K^{+}$ must diffuse from $V_{c\text{r}}$ through $V_{s}$ to reach the capillary space $V_{p}$. Although $V_{p}$ comprises the smallest volume, it is in reality more accessible for measurements of $[K^{+}]$ since $[K^{+}]_{p}$, closely mirrors $[K^{+}]_{c}$ (capillary plasma $[K^{+}]$) under conditions where blood flow is not occluded. The capillaries are usually perfused, which means that $K^{+}$ also enters this compartment from the arterial side. Thus both blood flow and the rate of exchange of $K^{+}$ across the capillary membrane markedly modify the total $K^{+}$ loss or gain by the muscle as a whole. Loss of $K^{+}$ from the active muscle is reflected in a rise of $[K^{+}]_{c}$, although in many studies $[K^{+}]_{c\text{mix}}$ has been measured. $[K^{+}]$ in the different compartments are further
influenced by the simultaneous water fluxes, which will be discussed separately. Finally, the processes in the subsequent recovery from muscle activity are summarized below. We have laid out the following section in a logical and linear sequence to illustrate these processes.

A. Release of $K^+$ From the Muscle Cell With Activation

Direct recordings from isolated muscle preparations in bathing solution have shown that each stimulation or AP is associated with a release of $K^+$ from the cells. The amount of $K^+$ released depends on the amplitude of the AP, any concurrent depolarizing currents, and the fiber surface area (including t tubules).

The first isotope studies applied $^{42}K^+$ loading of muscle tissue followed by subsequent measurements of unidirectional $^{42}K^+$ release and reported data of $\sim 11$ pmol/cm$^2$ (or about 17 $\mu$mol/kg wet wt) while the net release was only 7.4 $\mu$mol/kg wt wt$^{-1}$·stimulus$^{-1}$ during 2-Hz stimulation in the rat diaphragm at $38^\circ$C (124). Correspondingly, in single frog muscle fibers stimulated at a mean frequency of $\sim 2$ Hz (range 0.78–50), the unidirectional release rate was 11.4 pmol/cm$^2$·stimulus$^{-1}$ while the net release rate was 9.6 pmol/cm$^2$·stimulus$^{-1}$ at 21°C (299). The latter value corresponds to around 5 $\mu$mol/kg wt wt$^{-1}$·stimulus$^{-1}$. An alternative method to study unidirectional $K^+$ release is to block the Na$^+$-$K^+$ pump, since this is the main route for $K^+$ uptake. During ouabain block and stimulation at 1 Hz, a release of 9.4 $\mu$mol/kg wet wt was reported, but only a net $K^+$ release of 7.0 $\mu$mol/kg wet wt$^{-1}$·stimulus$^{-1}$ was measured in the absence of ouabain blockade in the rat soleus at 30°C (102, 182). Differences between species and type of muscle (heart, diaphragm, slow twitch or fast twitch, fiber size, or cross-sectional area) and hence surface membrane area per kilogram muscle wet weight may account for some of the variability (252, 264). At stimulation frequencies of 1–5 Hz, the $K^+$ release is in close agreement with a model of $K^+$ efflux predicting a release of 4 pmol/kg wet wt$^{-1}$·stimulus$^{-1}$ (270). However, the stimulation pattern may well affect the $K^+$ release associated with an AP.

Creese et al. (124) stimulated the diaphragm of rats at 5 Hz and calculated a unidirectional $K^+$ release of only 5 pmol/cm$^2$·stimulus$^{-1}$ or less than one-half the value obtained at 2 Hz. As an estimation of release per stimulus they preferred lower rates of stimulation because high stimulation rates caused a fall in twitch tension. Using a stimulation frequency of 400 stimuli/min or $\sim 7$ Hz for 30 min, a net release rate of only 1.3 $\mu$mol/kg wet wt$^{-1}$·stimulus$^{-1}$ was calculated (194). There may be several explanations for the decreased release per stimulus seen at higher stimulation frequencies, especially if stimulation is maintained for some time.

First of all, these studies have not shown that all stimuli were actually transmitted as AP. Furthermore, net $K^+$ release will induce increases in $[K^+]_m$ which have been monitored using ion-selective microelectrodes located in the interstitial space (229). A single AP can cause a local increase in $[K^+]$ on the muscle fiber surface from 2.5 to 4–5 mM, while during a train of 6 AP (delivered at a frequency of 10 Hz) the accumulated values were $\sim 10$ mM (295), and a 50-Hz stimulation for 20 s (1,000 stimulations) raised $[K^+]_m$ to only 8.8 mmol (297). In combination, the data indicate that the release of $K^+$ is not linearly related to the number of successive stimulations. Two different mechanisms may in combination account for this finding: a decrease in AP amplitude (see sect. IV C) and an activation of the Na$^+$-$K^+$ pump (see sects. IVB and VIB). Therefore, to quantify $K^+$ loss per impulse, $K^+$ release should ideally be recorded following only a single AP. It is due to the lack of sufficiently sensitive techniques that protocols are used in which the accumulated release from several stimulations is recorded.

Because $[K^+]_m$ is regarded as the single most important factor for muscle cell excitability, several authors have attempted to estimate the maximum rise of $[K^+]_m$. Naturally maximum $[K^+]_m$ must be at least as high as maximum $[K^+]_a$, and as argued in section VIB, the two are probably quite close in the homogeneously well-perfused muscle. However, $[K^+]_a$ almost 3 mM higher than $[K^+]_m$ has been reported (294). In this case, $[K^+]_m$ was measured at the surface of the muscle at which point it might differ from $[K^+]_a$ deeper inside the muscle. However, even when the electrode was inserted into the muscle, a 2.5 mM difference was reported (229), and such a difference between $[K^+]_a$ and $[K^+]_m$ may alternatively be due either to admixture of blood from other tissues or from perfused capillaries surrounding inactive fibers, i.e., heterogeneity of flow (215, 597, 614). The highest $[K^+]_m$ can be measured during high-intensity dynamic exercise. In humans a maximum average $[K^+]_m$ of 8.3 mM was measured at the end of 1 min of running, with several single measurements exceeding 9 mM (467, 468). During bicycling, slightly lower maximum values close to 8 mM were observed (462, 658). Interestingly, in thoroughbred horses, maximum $[K^+]_m$ values close to 9 mM have also been reported (262), and in experiments on muscles in rabbits, cats, and dogs, maximum $[K^+]_m$ of 7.5–8.2 mM have been measured (295, 636).

Various techniques have been employed to get a more direct estimate of $[K^+]_m$. Measurements in lymph and on the muscle surface underneath the fascia have provided estimates of maximum $[K^+]_m$ in the same range (294, 295, 636). Using microelectrodes inserted directly into the muscle, Vyskocil et al. (663) reported an average $[K^+]_m$ in the human brachioradialis muscle during maximum sustained isometric contraction of 9.5 mM with some measurements approaching 15 mM (663). During
such contraction, blood flow through the active muscle areas is occluded, and [K⁺]₀ may build up to values significantly higher than in [K⁺]ₑ. Interestingly, estimates based on [K⁺]₀ and [K⁺]ₑ during and after static contractions in humans also suggest values of [K⁺]ₑ in the order of 15 mM (555). In isolated mouse soleus muscles, Juell (329) reported a maximum [K⁺]ₑ of 9.9 mM using a technique with microelectrodes during repetitive stimulation in the nonperfused muscle (329).

More recently, the microdialysis technique has been used in humans (240, 334, 450). As mentioned in section II A, rupture of muscle cells will cause a rapid and large increment in [K⁺]ₑ. Therefore, all techniques that involve introduction of needles or hollow fibers into the muscle tissue are prone to artifactual measurements of the true [K⁺]ₑ but only the initial trauma after the insertion of, e.g., an electrode seems to cause leakage from injured muscle fibers (297, 663). During high-intensity knee extension exercise in humans, Juell et al. (334) recently reported an average peak [K⁺]ₑ of 9.04 mM using the microdialysis technique. There was great variability between different sites in the same muscle and between experiments with maximum observations approaching 14 mM. The authors conclude that there are large regional variations of [K⁺]ₑ within the exercising muscle. With the same exercise model, maximum [K⁺]ₑ has been reported to be lower (28, 31, 246, 253, 332, 595). Probably this discrepancy can be ascribed to dilution of the venous blood from exercising muscle with venous blood from less active muscle.

Using a different exercise protocol with stepwise increments in power instead of separate bouts at a given intensity, Green et al. (240) reported maximum [K⁺]ₑ with the microdialysis technique of 7.5 mM.

Two different approaches have been used to quantify release in the in situ or in vivo condition. As described in section II B and as mentioned above, an alternative to blocking the Na⁺-K⁺ pump to measure the outward diffusion rate (release rate) of K⁺ through K⁺ channels, \( J_{\text{Ksc}} \), is to measure the pump activity, \( J_{\text{Na-K pump}} \), as the unidirectional uptake of \( ^{42}\text{K} \) (598). Simultaneous measurements of the rate of intracellular K⁺ dissipation, \( \text{dK}^+ \cdot \text{dt}^{-1} \), allows calculation of \( J_{\text{Ksc}} \) from the flux equation

\[
J_{\text{Na-K pump}} + J_{\text{Ksc}} = \text{dK}^+ \cdot \text{dt}^{-1}.
\]

In these experiments twitch force was monitored throughout showing only minor changes, thus indicating that each stimulation elicited an AP (598). At rest, the K⁺ loss rate was not significantly different from zero, indicating that \( J_{\text{Na-K pump}} \) equaled \( J_{\text{Ksc}} \), both being in the order of 5 \( \mu\text{mol} \cdot \text{kg wet wt}^{-1} \cdot \text{s}^{-1} \). Within 45 s of 4-Hz stimulation, K⁺ loss, calculated from plasma flow and [K⁺]₀ reached a plateau of 1.2 \( \mu\text{mol} \cdot \text{kg wt}^{-1} \cdot \text{s}^{-1} \), while the \( ^{42}\text{K} \) uptake attained a value corresponding to a K⁺ uptake of 22 \( \mu\text{mol} \cdot \text{kg wt}^{-1} \cdot \text{s}^{-1} \). Thus K⁺ release during stimulation amounted to 23.2 \( \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{s}^{-1} \), corresponding to 5.8 \( \mu\text{mol} \cdot \text{kg wt}^{-1} \cdot \text{AP}^{-1} \) during 4-Hz stimulation, which tallies with the data above based on different techniques.

A quite different means by which to estimate the release from the cells is to measure the initial rate of rise of [K⁺]ₑ at onset of stimulation. Vøllestad et al. (658) and Hallén and co-workers (252, 254) used K⁺-selective electrodes in humans for continuous intravenous recordings of [K⁺]ₑ. They assumed \( [\text{K}^+]_s = [\text{K}^+]_e \), which can be justified in the well-perfused muscle as discussed above. In the “no-flow” state \( \text{dK}^+ \cdot \text{dt}^{-1} = -\text{dK}^+ \cdot \text{dt}^{-1} \) (assuming that activation of the Na⁺-K⁺ pump as well as co- and countertransport takes at least a few seconds). In this equation \( \text{dK}^+ \) is estimated from [K⁺]ₑ and interstitial volume. The extracellular H₂O was set to 15% of muscle wet weight, which may be a little on the high side for human muscle at rest, where it is 15–10% of total H₂O or 8–11% of muscle wet weight (5, 592, 600) and where the calculated K⁺ release rate will be correspondingly high. Interestingly, similar values on initial rate of [K⁺]ₑ of around 10 \( \mu\text{mol} \cdot \text{kg wt}^{-1} \cdot \text{s}^{-1} \) were obtained during knee extension (254) and bicycling (252), which is about one-half the observed value of the rat hindlimb stimulation study (598). For the rat study, the stimulation frequency was controlled, but for studies on voluntary contractions, calculations of K⁺ release per AP were based on an assumption of the actual firing rate. The firing pattern will occur in bursts of ~0.2-s duration every second, and a frequency estimate of 15–30 Hz has been inferred dependent on exercise intensity (252, 630). When combined, this results in a K⁺ release in the order of 2 \( \mu\text{mol} \cdot \text{kg wt}^{-1} \cdot \text{AP}^{-1} \). What is of note, however, is that the rate of rise was estimated from around seven bursts of four to nine AP, these bursts being separated by 0.8-s periods of no activity, where the Na⁺-K⁺ pump would pump K⁺ into the muscle cells at a steadily increasing rate. Intermittent activity pattern may therefore underestimate the K⁺ release per AP, but the rate of rise of [K⁺]ₑ was remarkably linear over several seconds in these experiments (658). By using K⁺-selective electrodes inserted into the muscle Hnï (295) showed values for rate of rise of [K⁺]ₑ around 100 \( \mu\text{mol} \cdot \text{kg wt}^{-1} \cdot \text{s}^{-1} \) with electrical stimulation at 50 Hz in cat and rabbit. This would correspond to a K⁺ release per AP of 2 \( \mu\text{mol} \cdot \text{kg wt}^{-1} \cdot \text{AP}^{-1} \), a value which is close to the 5–9 \( \mu\text{mol} \cdot \text{kg wt}^{-1} \cdot \text{AP}^{-1} \) found in rats, something which is particularly interesting when one considers that rat muscle fibers are small with a larger surface-to-volume ratio and that not all fibers are recruited during voluntary contractions as discussed in section II.

The linear relationship between muscle power output and rate of K⁺ release found by Vøllestad et al. (658) is in contrast to Creese et al. (124). This is because in the former study, release was only estimated immediately after onset of exercise so that none of the factors that attenuate K⁺ release with each AP during prolonged stim-
ulation periods was present. This tallies with Gebert (229) who, except at the highest frequency, found a linear relationship between stimulation frequency and peak $[K^+]_v$ after 15-s stimulation periods.

Sréter (616) noted that white muscle showed greater decrease in $K_m$ than red slow-twitch muscle during stimulation. At least part of this can probably be attributed to a larger release of $K^+$ for each AP in fast-twitch compared with slow-twitch muscle. In line with this, Nielsen and Clausen (495) showed that gain of $Na^+$ was several times faster in EDL compared with soleus at the same stimulation frequency. This is probably because the number of $Na^+$ channels and $Na^+$ current density is much higher in fast-twitch muscle (543). The implication of this finding is interesting, since it must mean that the inward $Na^+$ current is probably much larger than needed to depolarize the fibers. The overshoot of the action potential is only slightly higher in fast- compared with slow-twitch muscle fibers, and the former have slightly larger diameter than slow-twitch fibers (705). Hence, about the same amount of current is needed to charge the membrane capacitor and depolarize these two fiber types. The “extra” current passed in fast-twitch muscle can then only be sustained by a concurrent outward current as described in section IV. Possibly, there is a brief period during the AP in fast-twitch muscle cells where $Na^+$ and $K^+$ currents overlap, contributing to more pronounced changes in intracellular contents of these ions. This is of course not very efficient, but the advantage is a more rapid rise of the AP. It is also very interesting that the rate of $Na^+$ gain is much larger in muscles that are allowed to shorten compared with muscles that contract isometrically at the same stimulation frequency (496). Again, one might speculate that concurrent uptake of $Na^+$ and release of $K^+$ occur and that these currents are no longer separated in time.

The major $K^+$ release occurs through the delayed rectifier $K^+$ channels (see sect. III.A). However, recent evidence has been presented which suggests that, in addition, the ATP-sensitive $K^+$ channels ($K_{ATP}$ channels) are involved and that these may have dual effects on $K^+$ fluxes (405). Once opened, these channels will not close during the depolarization of the cell, and they will therefore contribute with an outward current throughout the AP. After the contractions cease, however, recovery is markedly delayed if the channels are blocked by glibenclamide, a finding that would be consistent with the notion that these channels may also pass an inward $K^+$ current provided the cells become hyperpolarized as outlined in section III.A. It is relevant for this interpretation that the $K^+$ conductance is increased fivefold in single frog fibers that have been stimulated to exhaustion (198) and that this increase is prevented by glibenclamide (91). Accordingly, addition of glibenclamide to severely fatigued fibers has been found to increase force (164). At present, it is not clear why these channels open in exhausted muscle, although several factors described in section III.A may contribute. Also, the $Ca^{2+}$-activated $K^+$ channels ($K_{Ca}$ channels) in the sarcolemma may contribute to the repolarizing $K^+$ currents as described in section III.A. The main effect of the open $K_{ATP}$ channels is probably an attenuation of the AP amplitude and reduced excitability, since the outward $K^+$ current will offset the inward $Na^+$ current. Therefore, opening of $K_{ATP}$ channels may not affect the $K^+$ release associated with an AP. However, the effect of opening $K_{ATP}$ channels on the $K^+$ release associated with AP has yet to be examined extensively; therefore, quantitative as well as qualitative data are speculative.

We conclude that under basal conditions $K^+$ release during one AP is in the range of 2–9 $\mu$mol/kg wet wt. The variation is primarily due to several phenomena. First, muscle fiber surface-to-volume ratio varies between species. Second, $K^+$ release per AP is probably higher in fast-twitch than in slow-twitch fibers. Third, $Na^+$ accumulation and hence probably also $K^+$ release is much greater during stimulation of muscles that are allowed to shorten compared with isometric contractions. Fourth, with maintained stimulation inward $Na^+$ current may go down so that the depolarizing current is diminished, and consequently, the repolarizing $K^+$ current also becomes smaller. We also conclude that the maximum $[K^+]_s$ attained in exercising muscle possibly can exceed 10 mM in localized regions. There is an amazing accordance between direct measurements of $[K^+]_s$ and attempts at more direct measurements of $[K^+]_s$ which both show that on average maximum $[K^+]_s$ is close to 9 mM for a well-perfused muscle working at high intensity.

B. Uptake of $K^+$ Across the Muscle Membrane

The primary mechanism for $K^+$ uptake is the $Na^+-K^+$ pump. Even in a quiescent muscle fiber, $Na^+-K^+$ pumping is required to compensate for the passive inward flux of $Na^+$ and associated loss of $K^+$ which are consequences of a basal leak (see sect. III.B). At rest, in isolated muscles only 2–6% of the $Na^+-K^+$ pump capacity is utilized. However, through the combination of intracellular $Na^+$ loading, increased apparent affinity for $Na^+$ and high extracellular $[K^+]$ the pump activity may reach around 90% of its theoretical maximum at 30°C (105). Such functional activity is assumed also to be available during exercise. In rat soleus muscle, the maximum activity allows for a $K^+$ uptake of $\sim 200$ $\mu$mol · kg wet wt$^{-1}$ · s$^{-1}$ (105). Combining this with a $K^+$ release of 6 $\mu$mol · kg wet wt$^{-1}$ · stimulus$^{-1}$ will allow for a maximum firing frequency of 30 Hz before the capacity of the pump is exceeded so that it no longer can keep up with the exercise-induced $K^+$ release (102). Based on this, Clausen and co-workers (103, 112) have pointed out that an inadequate $Na^+-K^+$ pump activity or
even inadequate maximum pump capacity may be one possible explanation for the lack of K\(^+\) homeostasis with exercise. However, sustained muscular activity at such high frequencies is rare in the in vivo condition.

During submaximal exercise the K\(^+\) loss that occurs from exercising muscles is largest in the beginning, although it may persist throughout the exercise. As described in section \(\text{mA}\), the transient nature of the loss of K\(^-\) from muscle after onset of activity is due to a delayed activation of the Na\(^+-K\(^+\) pump (252, 467, 567, 592). In the following we focus on measurements of uptake rates and on experiments that have tried to uncover the more important mechanisms that stimulate pump rate into compensating for the release.

Direct measurements of Na\(^+-K\(^+\) pump activity during muscle activity in rat was performed by perfusing the muscle with 42K\(^+\) as mentioned above (598). With a 4-Hz stimulation, pump activity increased from 5 to 22 \(\mu\text{mol} \cdot \text{kg wet wt}^{-1} \cdot \text{s}^{-1}\) or to \(\sim 10\%\) of its maximum rate or capacity, which in 4-wk-old rats is \(\sim 200 \, \mu\text{mol} \cdot \text{kg wet wt}^{-1} \cdot \text{s}^{-1}\) (102), but considerably lower in older rats (cf. Fig. 7). K\(^+\) uptake rates were also assessed in a series of experiments in humans based on the same principles as for estimating K\(^\text{mix}\) release per stimulus from the rate of [K\(^+\)]\(_c\) increase. The uptake rate was estimated from the initial decrease of [K\(^+\)]\(_c\) in the early recovery following exhaustion. These studies showed that just after high-intensity cycling (500 W) K\(^+\) uptake rate was close to 30 \(\mu\text{mol} \cdot \text{kg wet wt}^{-1} \cdot \text{s}^{-1}\) (658). Uptake rate was linearly related to muscle power output during the preceding exercise (252, 567, 658). Thus, with this kind of exercise, the Na\(^+-K\(^+\) pump may at the most be activated to a peak level of somewhat less than 50\% of the maximum rate, which in humans is \(\sim 70 \, \sim 80 \, \mu\text{mol} \cdot \text{kg wet wt}^{-1} \cdot \text{s}^{-1}\) (505). With a knee extension model, peak uptake rates proved to be somewhat smaller (246, 253). In this way, the maximum pump rate is probably never reached in these types of exercise if estimated as a mean for the whole muscle. However, K\(^+\) may not be taken up at equal rates by all fibers due to variable recruitment and firing rates. Interestingly, in these human experiments, initial release rates and uptake rates after several minutes of exercise matched almost perfectly, indicating that Na\(^+-K\(^+\) pump rates were well adjusted to the release. The uptake of K\(^+\) following exercise is summarized in section \(\text{mA}\).

As discussed in section \(\text{mA}\), the rise in [Na\(^+\)]\(_c\) that occurs with exercise (26, 183, 299, 329, 496) is insufficient to explain entirely the increase in Na\(^+-K\(^+\) pump rate unless some other type of activation of the enzyme occurs since even at a 20\% increase in [Na\(^+\)]\(_c\), a 5- to 10-fold increase in pump activity has been shown to occur (184, 496). Among the factors that can activate the pump in the sense that higher pump rates are achieved at the same [Na\(^+\)]\(_c\) are a number of hormones. The Na\(^+-K\(^+\) pump is stimulated by catecholamines, epinephrine being more effective than norepinephrine (430). However, \(\beta\)-adrenoceptor blockade has a relatively modest effect on the increase of plasma [K\(^+\)] during exercise (136, 343, 406), although in humans [K\(^+\)]\(_{\text{mix}}\) may be almost 1 mM higher at a given high-intensity exercise following propranolol treatment (86, 211, 243-245, 343, 406, 428, 562, 612, 645). Part of this effect of \(\beta\)-adrenoceptor blockade on [K\(^+\)]\(_{\text{mix}}\) has been ascribed to a diminished extracellular distribution volume (252), although this was not the case during exercise with a smaller muscle mass (knee extension) (246). Also, \(\beta\)-adrenoceptor blockade seems to increase release of K\(^+\) at a given exercise intensity (246, 252), although the reason for this remains unclear. It has been proposed that this effect may be due to an accentuated central drive that may be required for maintaining muscle power output. Finally, there is a small but definite effect of \(\beta\)-adrenoceptor blockade on the Na\(^+-K\(^+\) pump reuptake rates is gradually compensated for by a larger increase in [Na\(^+\)]\(_c\), that will counteract the inhibition. On cessation of exercise, therefore, there seems to be no detectable difference in rate of fall of [K\(^+\)], reflecting reuptake rates (246, 252).

Interestingly, the effect of \(\beta\)-adrenoceptor stimulation on Na\(^+-K\(^+\) pump activity seems to be somewhat different at rest and during activity. In isolated rat muscles, it has been shown that the effect of epinephrine is very much diminished in muscles that are stimulated and virtually nonexistent during intense stimulation (186). In exercising humans, the results are less clear since during many types of physical activity normally only some muscles are active while others are at or close to resting state. Thus, during one-legged knee extension, i.e., working with a quite limited muscle mass, administration of terbutaline, a \(\beta_2\)-adrenoceptor agonist, during steady-state exercise increased rather than decreased K\(^+\) release due to the fact that there was an increase in muscle blood flow while the [K\(^+\)]\(_{\text{mix}}\) remained unaffected (539). However, in resting humans, [K\(^+\)]\(_{\text{mix}}\) is rapidly reduced after administration of \(\beta\)-adrenoceptor agonists due to uptake in muscle (49, 540, 624), and in resting rat skeletal muscles, it has been convincingly shown that the pump is stimulated (106, 107). When exercise is started subsequent to administration of terbutaline reuptake, rates of K\(^+\) showed a lesser exercise-induced increase compared with the control situation (253). Thus it seems that Na\(^+-K\(^+\) pump rate is not only activated by catecholamines, but also by another factor at onset of exercise. Catecholamines have a small but still important effect on muscle K\(^+\) loss.

In addition to the circulating epinephrine and norepi-
nephrine released from the adrenal glands during exercise as described above, it is possible that the unmyelinated sympathetic axons that terminate directly on the muscle fibers (33) are another source of norepinephrine. The varicosities of these fibers could release transmitter after both increased sympathetic nerve activity in exercise and after passive depolarization by increased \([K^+]_s\) originating from the muscle fibers. Furthermore, there is evidence to suggest that CGRP, released from afferent nerve fibers (550) and from the motor nerve terminals, is another \(Na^+-K^+\) pump stimulant (20). The relative involvement of active and inactive muscle needs to be further elucidated, but obviously \([K^+]_{mix}\) is modulated by the effect of catecholamines on resting and contracting muscle cells, which supports the notion of \(K^+\) release from exercising muscle not normally being due to insufficient \(Na^-K^+\) pump capacity.

Given the discrepancy between the relatively small effects of hormones and the pronounced exercise-induced increase in \(Na^+-K^+\) pump rate far beyond that which can be ascribed to the rise of \([Na^+]_c\), another pump stimulant has been sought after. Interestingly, prolonged reduction of \([Na^+]_c\) below normal resting values may persist after the end of exercise, and in spite of this, the pump rate was increased above resting value (496). A stimulation of 60 Hz for 15 s at the point of decreased \([Na^+]_c\), elicited the same net \(Na^+\) extrusion as did this stimulation at normal resting condition, excluding \([Na^+]_c\) as the main pump stimulant (497). Most importantly, the excitation-induced accumulation of \([Na^+]_c\) and probably the \(K^+\) release depend highly on the mode of contraction; isometric contractions caused (5 times) less increase in \([Na^+]_c\) than shortening contractions without external force production, both in response to 10 s of electrical stimulation at 60 or 120 Hz (496). Correspondingly, \(Na^+\) efflux (reflecting \(Na^+-K^+\) pump rate) was markedly lower during isometric contractions than during shortening contractions. These findings point to hitherto unknown feed-forward mechanisms for stimulation of the \(Na^+-K^+\) pump as mentioned in section II.B.

In conclusion, \(Na^-K^+\) pump rate is greatly enhanced in exercising muscle in proportion to muscle power output. This stimulation has at least two components. The slow component is due to a gradual gain of cellular \(Na^+\) and a rise of \([Na^+]_c\). A small fraction of the more rapid component can be ascribed to catecholamines, whereas it seems that electrical activation by some intracellular mechanism triggers pump activation and thereby attenuates the effect of catecholamines.

C. Loss of \(K^+\) From the Muscle

Increased plasma \([K^+]\) with exercise has been reported in all species studied, such as trout (498), frog (63), rat (95), rabbit (636), cat (295), dog (294, 476, 500), sheep (320), calf (207) camel (553), horse, and human (467, 569, 592). Increased \([K^+]\) in arterial or mixed venous blood is indicative of \(K^+\) loss from the in situ exercising muscle. The loss of \(K^+\) from exercising muscle is the result of \(K^+\) release from the muscle cell surpassing \(K^+\) uptake, as discussed above, in combination with a subsequent wash-out of extracellularly accumulated \(K^+\) in the interstitial space. Direct quantification of \(K^+\) loss can be obtained from measurements of \([K^+]_a\) and \([K^+]_v\) together with blood flow over the exercising muscle or from analysis of \(K^+\) per unit of dry weight muscle tissue. Importantly, \(K^+\) is also lost by way of the lymph. The highest increment of \([K^+]_a\) (>10 mM) has been observed in exercising horses (261).

Many investigators use \([K^+]_{v-a}\) to calculate loss. As argued in section II, this is correct provided there is a steady state with regard to fluid balance, so that \(J_{Fa,in}\) equals \(J_{Fl,out} + J_{Fl, out}\) and assuming \([K^+]\) is equal in lymph and venous plasma. It is then necessary to use arterial plasma flow since lymph flow is usually not known. Some investigators have introduced a mathematical correction of \([K^+]_a\) that takes into account that venous hematocrit is higher than arterial hematocrit (462). The correction implies that the amount of \(K^+\) that enters the muscle in the arterial plasma is identical to the amount that leaves the muscle in venous plasma, the \([K^+]_v\) is set to zero even if a hemoconcentration has occurred during the passage through the muscle. Swelling of red blood cells will justify the correction. However, swelling of red blood cells across the vascular bed of an exercising muscle cannot be detected (246); on the contrary, a shrinkage has been reported by some investigators (422, 573). One deduced therefore that the hemoconcentration must be due either to uptake of fluid by the muscle (muscle swelling) or to lymph production. With onset of exercise there is indeed muscle swelling. The correction may also then be justified, since the calculation will reflect the true loss from the muscle. However, it is important to remember that a lot of \(K^+\) accumulates in the interstitium of the muscle during this period, which means that the dissipation of \(K^+\) may greatly exceed the total muscle loss. Finally, during a steady-state situation, the correction is not justified since a large lymph production was calculated to have occurred assuming constant muscle water content (246). Hence, the switch from loss to gain of whole muscle \(K^+\) that McKenna et al. (462) claim takes place at 1 min after onset of high-intensity bicycling and the maintained gain throughout the rest of the exercise period is probably not correct. Interestingly, Gullestad et al. (246) estimated that during knee extension exercise lymph production was ~70–80 ml/min from one leg or ~4% of the plasma flow. With the observed \([K^+]_{v-a}\), the lymph flowing at this rate would contain as much \(K^+\) as is actually lost from this muscle. This may be
further accentuated since $[K^+]$ in the lymph may be higher than in the vein after exercise (636).

1. Intensity and mode of exercise

Muscular activity or power output may be classified according to the pattern of the electrical stimuli and/or the mechanical response. Contractions may be voluntary or stimulated, intermittent (with a given frequency determining the duty cycle) or continuous. Depending on whether force is exerted during muscle shortening, lengthening or constant length, contractions are classified as concentric, eccentric (both of which are dynamic), and static (or isometric). The muscle is creating power in a physical sense only during dynamic concentric activity. Power output is also referred to as work load, exercise intensity, or physical activity, and these terms will be used corresponding to the cited publications. The stimulation pattern is of major importance for the $K^+$ loss from the active muscle. This is well controlled during in vitro or in vivo stimulation experiments, but only delicate techniques allow for measurements of the pattern of AP from a few motor units at a time during voluntary contractions, and data during dynamic contractions are especially rare. Also, the mechanical response affects $K^+$ loss both through a direct effect as recently shown (496), and more importantly through the effect of muscle contractions on blood and lymph flow. These parameters are rarely given in sufficient detail to allow comparisons between studies.

Finally, active muscle mass determines the amount of $K^+$ transferred from the active muscles to the extracellular space and other tissues. What is interesting is that the active muscle mass seems to be an independent determinant of the $K^+$ loss from the muscles, possibly through a central feedback mechanism as proposed by Hallén (251). The most common modes studied in humans are running, bicycling, knee extension, and hand-grip exercise, here mentioned in order of decreasing active muscle mass and where static as well as dynamic exercise may be performed in the two latter models.

During static contraction at high force, no $K^+$ is lost from the muscle to the circulation because there is no perfusion. The intramuscular pressure increases with contraction intensity and impairs or occludes blood flow when it approaches or surpasses blood pressure (554, 568, 597). During sustained static contraction, the intramuscular pressure increase persists as long as the contraction is maintained, while during repetitive static as well as during dynamic contractions, intramuscular pressure decreases to resting value between contractions. During the sustained contractions, blood flow may be completely occluded when contraction force approaches 50% maximum voluntary contraction force (MVC), depending on muscle group and fiber composition. In contrast, during the repeated static or dynamic contractions, periods with high blood flow are seen between the contractions compensating for insufficient flow during the contractions (75, 535, 602, 661).

Kjellmer (355) showed in a cat muscle preparation that $[K^+]_v$ and muscle flow rise in parallel during stimulation. Tibes et al. (638) were among the first to present simultaneous measurements of leg blood flow and plasma $[K^+]$ in humans and showed simultaneous increases in both variables with increasing exercise intensity. However, they only measured $[K^+]_v$, and not $[K^+]_o$; therefore, they could not calculate the $K^+$ loss although their data were indicative of increases in $K^+$ loss with increased exercise intensity.

As mentioned above, increases in $[K^+]_v$ or $[K^+]_{v, mix}$ are only indicative of a $K^+$ loss from the exercising muscle. Most often in whole body exercises the $[K^+]_{o, v}$ is so small that $[K^+]_o$, $[K^+]_v$, or $[K^+]_{v, mix}$ give roughly the same information about the $K^+$ content of the extracellular space, $V_v$. In humans, the highest plasma $[K^+]$ of $\sim$9 mM was reported in high-intensity 1-min exhaustive running, e.g., whole body dynamic exercise involving leg and also to some extent upper body/arm muscles (467, 468). During submaximal running at steady-state level ranging from 30 to 90% maximum oxygen consumption ($\dot{V}O_{2, max}$), $[K^+]_{v, mix}$ increased in proportion to running intensity from 4.6 to 6.0 mM (72, 73, 700). A similar pattern is reported for bicycling, where upper body muscles are activated to a somewhat lesser degree. Also here $[K^+]_{v, mix}$ correlates closely with work load or oxygen uptake (638, 658, 713), although the peak $[K^+]_v$ in the femoral vein was slightly lower than during running: $\sim$8 mM at bicycling to exhaustion at 110% of $\dot{V}O_{2, max}$ (Fig. 1) (658). In most other bicycle protocols, much lower values are reported: 4.6–5.8 mM (425, 442, 589, 638); however, the studies are not entirely comparable because of varying exercise intensities, protocols, and times of sampling. When only the knee extensors were studied, even lower peak $[K^+]_v$ values were attained during maximal intensities: $\sim$7 mM (332, 595), but again a close relationship to exercise intensity was seen (555). An extensive number of studies unanimously confirm the increase in either arterial, mixed venous, or femoral venous $[K^+]$ with increasing dynamic exercise intensity and are summarized in several reviews (408, 421, 592, 594).

The scenario of the time history for changes in $[K^+]_v$ is as follows: after onset of exercise, the rate of rise in $[K^+]_v$ is higher the more intense the exercise (252, 658) (see Fig. 9). At maximal dynamic exercise intensities, $[K^+]_v$ increases until exercise is terminated at exhaustion (467), whereas during submaximal exercise, a peak level is usually attained within a few minutes (658). Subsequently, $[K^+]_v$ will level off to some plateau below the peak and depending on the intensity will remain at this level or tend to further decrease or increase later during the exercise. The peak is most prominent in the effluent
venous blood, $[K^+]_v$, but may even be visible in the arterial concentration, $[K^+]_a$, with the latter initially being always lower than $[K^+]_v$.

The $[K^+]_{v-a}$ remained high throughout bicycling at 110–140% $\dot{V}O_2\text{max}$ but was zero later during intensities of 60–85% $\dot{V}O_2\text{max}$ (658). This is corroborated by another study in which bicycling at 75% $\dot{V}O_2\text{max}$ for 50 min increased $[K^+]_{v-a}$ to 0.5 mM within 1 min, but $[K^+]_{v-a}$ was not significantly different from zero after 2–10 min of bicycling, depending on the muscle glycogen level (422). However, after 20 min of bicycling at 60–80% $\dot{V}O_2\text{max}$, a positive $[K^+]_{v-a}$ was found in the order 0.05–0.2 mM, which was maintained throughout the exercise (549). In this study no samples were obtained early during exercise, and it is quite possible that loss is transiently reduced almost to nil, but reappears if exercise is continued (658). In the glycogen-depleted state, muscle $K_c^+$ may be reduced (43, 45), which may result in an attenuated $K^+$ loss (422). The mechanism for this may be a changed recruitment of motor units due to glycogen depletion of some muscle fibers. Especially in the case where fast-twitch fibers have become glycogen depleted, an increased number of slow-twitch fibers may be activated, and these slow-twitch fibers have a larger capacity to maintain $K^+$ homeostasis. In line with this, $[K^+]_{v-mix}$ tended to be higher in subjects during standard bicycling tests after glycogen repletion compared with a glycogen-depleted condition (74). However, such glycogen dependency has not been confirmed during knee-extension exercise (28).

During one-legged intense knee extension, the effluent $[K^+]_v$ was ~6.0–6.8 mM, and the $[K^+]_a$ was 5.3–5.8 mM (28, 31, 332, 595). These values are lower than those during whole body exercise, although the $[K^+]_{v-a}$ values are generally larger, with the difference first of all being due to the smaller active muscle mass in the knee-extensor model. Less total $K^+$ is lost, and $[K^+]_a$ does not rise as much. In addition, the volume for redistribution of $K^+$ is slightly larger. Interestingly, a $[K^+]_{v-a}$ statistically significantly different from zero was found during all exercise intensities of knee extension, even as low as 5% MVC, and the $K^+$ loss was not abolished even during prolonged exercises of 1–2 h (592). All studies report that $[K^+]_{v-a}$ remains positive during knee extension (28, 29, 31, 332, 345, 346, 539, 590, 591, 595, 602). It is unclear why there is a maintained positive $[K^+]_{v-a}$ during knee extension, whereas during bicycling the $[K^+]_{v-a}$ may disappear. However, it does fit with a smaller reduction of $K_m^+$ per kilogram dry weight during whole body (44, 45, 589) compared with knee-extension exercise (592). Furthermore, a study comparing rat swimming with electrical stimulation of perfused rat hindlimb clearly demonstrated significantly larger decreases in muscle $K_m^+$ in the latter case, which was true for red and white portions of the hindlimb muscles (418). These findings reflect an insufficient stimulation of the $Na^+-K^+$ pump (655). A relatively larger blood flow per kilogram active muscle mass is reported during knee extension (19, 346), but there is no reason why $Na^+-K^+$ pump activation should be flow dependent. The lower $[K^+]_a$ is probably not important either, as the extracellular site of the pump is close to saturation at normal $[K^+]_a$. Hallén (251) proposed that the lower sympatoadrenal activation during exercise with a limited muscle mass could be an explanation.

The highest rate of $K^+$ loss occurred during high-intensity dynamic contractions, which the subjects could only continue for a few minutes and was in the order of 1 mmol kg$^{-1}$ wet wt$^{-1}$ min$^{-1}$ estimated over the full exercise period (332, 595). Initial rates of dissipation of $K^+$ are much higher than the loss rate from the whole muscle averaged for the entire exercise period for two reasons. First, the $Na^+-K^+$ pump is rapidly activated. Second, as outlined in section aB, loss only reflects the mismatch between release and uptake at the cell level provided the interstitial content $K_m^+$ does not change. For instance, $K^+$ loss rates from the whole muscle of this magnitude are...
reported by several investigators (31, 332, 595) after 30–60 s of exercise. However, during the same time period, $[K^+]_i$ increased by up to 2 mM, reflecting a similar build-up of $K^+$ in the interstitium. In other words, these loss rates may underestimate the true net dissipation of $K^+$ by 30–50% (252, 658). However, when a stable $[K^+]_i$ is reached, this error is minimal. The gradual accumulation of $K^+$ in the interstitium is reflected in the finding that loss from the whole muscle peaks after 1–2 min, whereas peak net release from muscle cells is maximum at onset of activity (see sect. III).

The largest observed total $K^+$ loss occurred during prolonged dynamic knee extension at submaximal intensity which could be maintained for 2 h or more before exhaustion occurred, and amounted to $\sim$20 mmol/kg wet wt (590). This estimate of total loss was obtained by analysis of tissue samples on a dry weight muscle tissue basis. After intense dynamic exercise, a 10% decrease of $K_m^+$ was seen; however, after prolonged exercise up to 20%, $K_m^+$ may be lost from the muscle, i.e., a decrease from 485 mmol/kg dry wt at rest to 431 mmol/kg dry wt after 2-h exercise at 60% $V_{O_2,max}$ (590, 595). A radioisotope study using $^{42}K^+$ during single leg extension, however, only detected a 3% decrease, but a number of methodological errors may contribute to the discrepancy (523). Interestingly, with exhaustive bicycle exercise, only small or insignificant reductions in $K_m^+$ analyzed on muscle biopsies per kilogram dry weight muscle tissue have been reported (44–46, 589). Only one study of intense bicycle exercise reported a large decrease of muscle $K_m^+$ from 410 to 355 mmol/kg dry wt (420).

A few studies have reported $K^+$ loss from static contractions in the range of 5–50% MVC in the knee-extensor muscles (554, 591) and forearm muscles (75–77, 189, 322, 602). Due to the restriction in blood flow, especially at the high contraction levels, these data on $K^+$ loss will invariably underestimate the release from the cells, since accumulation of $K^+$ in the interstitial space will occur as pointed out previously (555, 599). The general finding is that the largest $K^+$ loss occurs at low prolonged contractions and is essentially nil at high contraction intensities that can only be maintained in the order of 1 min. Interestingly, when corresponding tension-time contractions were performed as either sustained or intermittent (5-s contraction, 5-s relaxation), then $K^+$ loss was two to five times larger in the latter case (602). Manipulations with contraction intensity, duration, and duty cycle may eventually result in a protocol, where $K^+$ loss is zero due to uptake in each rest period, as shown for overall data during low-frequency (2 Hz for up to 6 hrs) or low-intensity intermittent stimulation (45-s stimulation and 15-s rest) of rat soleus muscles (616).

The above data on loss of $K^+$ relative to exercise intensity cannot easily be compared with data from stimulated muscle preparations, since it is difficult to normalize for stimulation frequency or muscle weight. Even so, an attempt has been made to recalculate some of the data relative to estimated numbers of AP or per contraction (593). These results must be taken with caution due to the number of assumptions as well as not accounting for the changes over time, although these rough calculations show that the techniques available for studying electrolyte translocations in human voluntary exercise are sufficiently reliable. Interestingly, recent studies have applied electrical stimulation of the knee extensors, and they could mimic voluntary dynamic contractions by a stimulation pattern of 50 Hz for 320 ms every second giving 16 stimulations/contraction and 960 stimuli/min (345). For corresponding work intensities, the arterial and venous $[K^+]_v$ were similar, although the flow and other metabolic responses were a little higher during stimulation (346). Taken all together, however, the estimates of net losses of 1–2 $\mu$mol·kg$^{-1}$·stimulation$^{-1}$ or 15–17 $\mu$mol·kg$^{-1}$·min$^{-1}$·contraction$^{-1}$ during voluntary dynamic contractions are confirmed by these stimulation studies.

In conclusion, activity-induced $K^+$ release from muscle cells may result in muscle $K^+$ loss only in a perfused muscle. Blood flow is occluded during high-force static contractions and in line with this $K^+$ loss is nil, whereas some $K^+$ loss may occur at low forces during static contractions. During dynamic contractions, the main determinant for the rate of $K^+$ loss is the magnitude of the power output. The $K^+$ loss rate is largest within the first minutes of the exercise period but may persist throughout activity, which is the general finding during exercise with a limited muscle mass such as the knee extensors. During whole body exercises, however, $K^+$ loss may transiently become nil. The largest total $K^+$ loss corresponding to 20% of resting $K^+$ seems to occur with prolonged dynamic knee extension.

2. State of training

The earliest study on the effect of training regarding muscle electrolytes dates back to 1936, where an increased $[K^+]_v$ of up to 10% in the skeletal muscles of trained rabbits was reported (69). For more references consult section v. Furthermore, with exhaustive exercise, trained rats decreased their muscle $K_m^+$ to lower levels than the untrained. Nöcker et al. (499) indicate that this might be associated with the better performance of these rats. Recordings of plasma $[K^+]_v$ in calves showed that at the same exercise intensity the $[K^+]_v$ increase was 5% smaller after training (207), which compares well with a significantly smaller rise in $[K^+]_v$ in endurance-trained compared with untrained subjects exercising at the same absolute power (454). Consequently, in the trained state, exercise could be sustained at much higher muscle power while their maximal $[K^+]_v$ rose to the same or only slightly higher than pretraining maximal values (207, 454).
Similarly, it has been reported that $K^+$ loss at a given submaximal exercise level was lower after 4 wk of knee-extensor endurance training (344). Also, when athletes were working at the same relative intensity (75% $\dot{V}O_2_{\text{max}}$) after 4 wk of detraining, $[K^+]_{c_{\text{mix}}}$ values were similar (440). In line with this, a number of cross-sectional studies on controls compared with athletes (endurance or sprint trained) have reported the following: $[K^+]_{c_{\text{mix}}}$ is higher in the untrained than in the trained at a given oxygen uptake but similar when expressed in relation to %$\dot{V}O_2_{\text{max}}$ (637, 638); the trained showed the highest maximal $[K^+]_{c_{\text{mix}}}$ at their higher maximal working loads (442). Those who were sprint trained attained higher $[K^+]_{c}$ than those who were endurance trained as the sprinters could run at a higher speed (468). In one longitudinal study, $[K^+]_{c}$ increased to 5.4 mM during maximal exercise in the control condition but to only 4.8 mM after 3 wk of bed rest and to 5.6 mM after 8 wk of subsequent training. All measurements were done at the same oxygen uptake, which probably corresponds to a higher relative exercise intensity in the detrained condition (552). In heart failure patients, the rise of $[K^+]_{c}$ was reduced in proportion to maximum exercise intensity (628). In short, $[K^+]_{c}$ and $[K^+]_{c_{\text{mix}}}$ rise in relation to the level of exertion, and the $[K^+]$ increase has been suggested as an objective measurement of relative work load (73). Because trained subjects can voluntarily accept higher levels of exertion, they can also attain the highest $[K^+]_{c_{\text{mix}}}$ levels. For a comprehensive review on this matter, see McKenna (461).

When exercising at a given absolute work load, however, the above-cited studies seem to document that a trained muscle loses less $K^+$ per kilogram wet weight. We suggest that the reasons for this may be several: with some types of training, muscle mass increases due to hypertrophy of the single fibers. Hence, fewer motor units have to be activated to provide the muscle power. In addition, the surface-to-volume ratio of these cells is decreased. Both these factors will reduce the $K^+$ release required to attain a given muscle power. However, also with endurance training which does not lead to hypertrophy, muscle $K^+$ loss for a given muscle power is reduced (344), which could be related to improved motor control. As outlined in section V, several studies have shown that the $Na^+\text--K^+$ pump concentration in muscle is increased with training. Thus uptake capacity is also increased, although it remains to be seen to what extent training affects pump activation. Finally, it may be important in this respect that $[K^+]$ plays an essential role as a biofeedback signal for cardiorespiratory regulation as discussed by Sjøgaard and co-workers (592, 599).

In conclusion, training attenuates $K^+$ loss from the exercising muscle at a given absolute muscle power output. It is possible that a small, yet significant upregulation of the $Na^+\text--K^+$ pump concentration in muscles contributes to this. On the other hand, it seems that in relation to relative work load, the steady-state $[K^+]$ in the general circulation is not affected by training. Interestingly, it seems that during ongoing exercise there is no tendency toward bringing $[K^+]$ in the general circulation back to resting values, but that instead it is maintained at a steady level depending on the relative work load.

3. Muscle fiber types

In the resting rat and guinea pig muscles, differences in ionic compositions have been reported, with the fast-twitch muscles having higher $K^+$ and lower $Na^+$ content and cytosolic concentrations $[K^+]_c$ and $[Na^+]_c$ (84, 95, 520, 619). In human muscle, soleus, vastus lateralis, and triceps brachii, the same tendency of differences was seen between single slow-twitch and fast-twitch fibers, although the differences were somewhat smaller (only ~5%) and could not be proven statistically significant on the limited material (589). The lower $[K^+]$, probably explains the less negative resting $E_m$ in slow-twitch fibers compared with fast-twitch fibers (81). When rat muscles are stimulated with identical activation patterns, the net electrolyte exchanges over time are different as well. This has been studied based on direct analysis of total muscle tissue electrolyte content following different stimulation regimes (414, 418, 484, 616). Low stimulation frequencies (<2 Hz) induced no decrease in $[K^+]_c$ nor increase in $[Na^+]_c$ in the slow-twitch soleus muscle, whereas large decreases in $[K^+]_c$ and increase in $[Na^+]_c$ were seen in the predominantly fast-twitch muscles plantaris and white gastrocnemius (616). At higher stimulation frequencies, this difference was attenuated, but was still present at tetanic stimulations (414, 418). Interestingly, fast-twitch muscle could maintain unchange $K^+$ when stimulated intermittently with 15-s rest periods (616). A quite extensive study (484) demonstrated that in rat muscles the largest differences were elicited at stimulation frequencies from 10–20 Hz and confirmed the general finding that slow-twitch muscle only lose $K^+$ during high stimulation frequencies, whereas fast-twitch muscles lose $K^+$ even at low frequencies. The reasons for these differences reside in the density of the $Na^+\text--K^+$ current and number of $Na^+\text--K^+$ pumps as discussed above and in section II B. With increased stimulation frequency, a smaller release per stimulus was calculated from the data by Sréter (616) on the rat white gastrocnemius, where increasing the stimulation frequency from 5 to 200 Hz decreases the release per stimulus by two orders of magnitude. Also, during more prolonged stimulation of up to 24-h duration, larger differences by fiber type are found when the $K^+$ loss from rat soleus was minimal, although a statistically significant (~24%) decrease was found in the EDL and in the tibialis anterior muscle (185). This finding may be surprising since the EDL is composed
mainly of fast-twitch fibers, which have higher Na\(^+\)-K\(^+\) pump concentrations (see sect. v). However, as discussed above, these fibers also have a higher Na\(^+\) current density.

In conclusion, K\(^+\) loss is larger from fast-twitch white muscles than from slow-twitch red muscles during continuous stimulation, especially at low stimulation frequencies where slow-twitch muscles may maintain K\(_m\) unchanged for prolonged time. However, fast-twitch muscles are more resistant to intermittent stimulation than are slow-twitch muscles.

D. Redistribution to Other Tissues

The redistribution of K\(^+\) with knee-extension exercise and bicycle exercise has been reviewed previously (251, 555, 567), and we here focus on some main aspects. With short-lasting exercise, K\(^+\) lost from the active muscles is primarily mixed within the available extracellular space. During one short exercise bout, for instance 1 min and less, the available redistribution volume is probably not much larger than the plasma volume due to low perfusion of some tissues. With increasing exercise time, other remote tissues and resting muscles are gradually perfused with arterial blood with high [K\(^+\)], and K\(^+\) also diffuses into the interstitial space of these tissues and will then be taken up by the cells. Thus redistribution is highly time dependent, and with short-lasting exercise, steady state will not be reached. In some contrast to this argument, Lindinger and co-workers (416, 420) argue that an extensive redistribution of K\(^+\) within the extracellular space and also into cells of remote tissues can take place even during very short-lasting exercise (30 s). However, their conclusion is based on an estimate of K\(^+\) loss from the active muscle that is more than 10 times higher than any other reported value (252, 332, 539, 658). They base their calculation on muscle biopsy data that have a large inherent variation. It is also clear that maximum rates of K\(^+\) loss from the exercising muscle reach a maximum with a delay of almost 2 min (332), since initially K\(^+\) accumulates within the interstitial space inside the muscle as outlined in section iiC. Thus extensive redistribution will not take place during very short-lasting exercise.

In addition to a time-dependent increase of available extracellular space, redistribution is also dependent on the absolute size of this space. Interestingly, the available extracellular space is dependent on exercise intensity and is also influenced by catecholamines. For instance, Hallén et al. (252) noted that the same loss of K\(^+\) from the legs during cycling at low exercise intensity resulted in a much smaller rise of [K\(^+\)]\(_m\) than at high intensity, indicating that the available mixing volume was smaller at high intensity. This can also partly explain the curvilinear relationship between exercise intensity and rise of [K\(^+\)]\(_m\) (467, 567, 658). In the same study (252), it was also shown that although loss of K\(^+\) from the muscle was not affected by \(\beta\)-adrenoceptor blockade during increments in power at high intensities, the accompanying rise of [K\(^+\)]\(_m\) was higher after the drug, showing that \(\beta\)-adrenoceptor blockade reduced the available redistribution volume. With knee extension exercise, engaging a smaller muscle mass and probably less sympathoadrenergic stimulation, the same effect on available redistribution volume was not observed during \(\beta\)-adrenergic blockade (246).

With increasing time, K\(^+\) is also taken up by the cells of the remote tissues. Two factors seem to be important for the uptake rate, namely, a rise of [K\(^+\)]\(_m\) which to some extent can stimulate the Na\(^+\)-K\(^+\) pump and reduce conductance of IRK, and hormonal control of the Na\(^+\)-K\(^+\) pump of the remote tissues. The kinetics of redistribution of K\(^+\) are therefore very different with prolonged exercise, and a quasi-steady state may be reached when net loss rate of K\(^+\) from the exercising muscle balances the rate of redistribution. Exercising for 2 h at intensities of 50–70% \(\dot{V}_{\text{O}_2}\) max with the knee extensors resulted in a K\(^+\) loss of 50 nmol K\(^+\) (590), which would have increased the [K\(^+\)] in the total extracellular space including \(V_p\) by more than 5 mM according to calculations based on assumptions of water distribution spaces as summarized in section \(\text{mm}E\) (601). However, [K\(^+\)]\(_m\) remained at a constant level of close to 5.0 mM while [K\(^+\)]\(_a\) stayed close to 5.2 mM throughout the exercise, indicating also only a limited increase in [K\(^+\)]\(_a\) of \(\sim 1\) mM (590, 601). Corresponding estimates of [K\(^+\)]\(_a\) with repeated high-intensity bicycle exercise bouts reach theoretical values for total extracellular [K\(^+\)] around 30 mM, whereas in these studies measured [K\(^+\)]\(_m\) never attained values higher than 5.5–6.3 mM (417, 420, 592). These discrepancies can only be explained by an excretion by the kidneys or an uptake of K\(^+\) in remote tissues, e.g., resting muscle. The latter was documented by negative values of [K\(^+\)]\(_v\) across the resting leg during one-legged knee-extension or arm exercise (30, 539, 590) and across the arms during bicycle leg exercise (416).

In studies with one-legged knee-extension exercise, biopsies were also taken from the inactive leg, but an increase in K\(_m\) could not be confirmed (590). Also, after repeated 30-s bicycle bouts, no change was observed in K\(_m\) of the deltoid muscle (416). Exercising with only the knee extensors of one leg means that only 3 kg or \(\sim 10\)% of the muscle mass is active, whereas 27 kg of muscles may take up the K\(^+\) loss. Estimates show that this would imply a relatively small increase of \(< 10\)% of total resting muscle K\(_m\), which in combination with relatively large biological variations in small muscle samples makes it difficult to measure. However, with the repeated 30-s bicycle bouts, much more muscle was engaged in the exercise, and the lack of detectable uptake in the resting deltoid muscle is probably due to the limited exercise duration. Thus a unilateral stimulation sustained for 6 h in
the rat caused a small but detectable increase in $K^+$ in the contralateral resting muscles (616).

Net uptake of $K^+$ into cells in a resting individual is a slow process as outlined in section II. Therefore, the activation of the sympathoadrenergic system during exercise is important for speeding up this process by stimulating the Na$^+$.K$^+$ pump in resting muscle cells. This is probably another reason why $\beta$-adrenoeceptor blockade causes higher $[K^+]_a$ or $[K^+]_{v \text{-mix}}$ during exercise (243, 244, 246, 252, 567). Indeed, $\beta$-adrenoeceptor blockade was shown to decrease $K^+$ uptake by inactive tissue (343). In a recent study Juel et al. (333) show that even exercising muscle can switch from a situation of net $K^+$ loss to net $K^+$ gain. During knee extension exercise that caused a stable net $K^+$ loss from the legs, the subjects started to perform arm exercise. This caused $[K^+]_a$ to increase further due to the additional net loss of $K^+$ from the arms. Interestingly, within 3.5 min of arm exercise, the $[K^+]_{v \text{-a}}$ across the still exercising legs switched from positive to negative. Actually, net $K^+$ uptake rates in the exercising legs were higher than the preceding loss rate. The authors explain this switch by the further increase in circulating catecholamines that occurred with the addition of arm exercise. However, the rise of $[K^+]_a$ in the exercising leg will also affect the IRK channels, and it is also possible that $K^+$ release was reduced.

Conflicting data have been reported on the role of the red blood cells regarding redistribution of $K^+$. In one early study both red cell volume and intracellular red cell $[K^+]$ ([K$^+]_a) of blood sampled from the femoral vein remained constant during bicycle exercise of increasing intensity despite marked acidosis at the highest intensities (67). This is in line with the unchanged arterial as well as venous $[K^+]_a$ during knee-extension exercise (539). However, volume changes of red blood cells seem to occur during and after several types of exercise. Most often a small and sometimes transient red cell shrinkage of a few percent occur during exercise, and a slight transient swelling can be observed in the recovery period (332, 419, 573, 658). It is therefore important to relate alterations in red cell $K^+$ content ($K^+_r$) to red cell hemoglobin content and not to red cell volume. When this is done, several studies fail to find any changes in $K^+_r$ during exercise (332, 333, 419, 437, 658). In contrast to this, in experiments with repeated intense 30-s bicycle bouts, the brachial artery $[K^+]_a$ was reported to increase while at the same time the femoral venous $[K^+]_a$ did not change. In both the femoral venous and the arterial blood samples, plasma $[K^+]$ increased by the same amount (417, 459). The reported $K^+$ uptake into arterial red blood cells was of the same order of magnitude as the increase in the plasma (63 mM each), and this was suggested as a means to keeping $[K^+]_{v \text{-mix}}$ low (459). However, quantitatively the capacity of the red blood cells compared with other inactive tissues is quite limited, particularly when taking into account that, for example, $>50$ mmol $K^+$ may be released from the knee extensors and removed from the circulation by uptake in other tissues of which the red blood cells only comprise a minor fraction.

Two studies indicate that exercise might lead to enhanced $K^+$ fluxes across the red cell membrane, especially due to activation of the red cell Na$^+$.K$^+$ pump (419, 437). However, increased fluxes do not necessarily mean that $K^+_r$ will change. An increased Na$^+$.K$^+$ pumping could be accompanied by an increased $K^+$ leak from the cells. It could also be speculated that $K^+$ uptake by red blood cells could be a function of the training status, but red cell Na$^+$.K$^+$ pump activity was similar between well-trained athletes and sedentary subjects (282). Altogether, we find it unlikely that red blood cells contribute significantly to removing $K^+$ from the circulation during exercise.

One consequence of $K^+$ being accumulated remote from the exercising muscle is that it is no longer available for immediate reuptake into the fatiguing muscle fibers, and the recovery process may be delayed for this reason (76, 592) (see sect. vnF). A very important consideration is therefore that the enhanced uptake affects not only fibers in resting muscle but also quiescent ones in the exercising muscle itself. Thus, in half-maximal contractions, pump-induced hyperpolarizations were found that were as prominent in the relaxed fibers as in the active ones (376). For further discussion, see Reference 599.

In conclusion, redistribution of $K^+$ lost from the active muscles has several components with very different time constants. Complete mixing in the available extracellular space may take time due to low perfusion of remote tissues. Both exercise intensity and sympathoadrenergic activation are determinants of the mixing time constants and accessible mixing volume. $K^+$ uptake into the cells of the remote tissues is probably accelerated by the sympathoadrenergic stimulation that occurs.

### E. Fluid Movements Between Various Spaces

$K^+$ is distributed in the water spaces of the body with high intracellular and low extracellular concentrations, changes in both of which depend on $K^+$ exchanges as well as water fluxes. The human body is composed of 50–60% water, which corresponds to 70% of lean body mass being water, a total of $\sim$40 l. Of these 40 l, $\sim$25 l at rest are located in the intracellular compartments $V_c$, whereas 15 l are located extracellularly, with 3 l plasma volume $V_p$, and 12 l in the interstitial space $V_i$. Red blood cell volume is $\sim$2 l. Skeletal muscle amounts to $\sim$40% of body weight or $\sim$30 kg, of which in the resting condition 75% is water, accounting for around one-half the body water. The distribution of total muscle water at rest is as follows: 90% (20 l) in $V_c$, 9% (2 l) in $V_i$, and 1% (0.2 l) in $V_p$. 

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The fluid distribution volumes are substantially changed during muscular activity. During exercise, there is an acute uptake of fluid by the active muscle cells as described in section II.D. Increased interstitial oncotic pressure and increased capillary hydrostatic pressure will subsequently or simultaneously cause net flux of fluid from the plasma into the interstitial space. The relative distribution of the fluid between $V_c$ and $V_s$ is subject to variation related to exercise intensity and duration.

In the perfused muscle of animals, the fluid distribution changes over time were monitored in different muscle fiber types and with different stimulation regimes (414, 418, 616, 617, 636, 669). The largest increase occurs early during activity. Fast-twitch muscle was most susceptible to volume increase, the major part of fluid being transferred to $V_c$. With prolonged stimulation, up to several hours, a subsequent decrease may occur. Interestingly, restricted motor activity over prolonged period of time, i.e., up to 90 days, caused a decrease in extracellular water from 62 to 48 ml/kg wet wt.

In the human during submaximal (50–70% $V_{O_2\text{max}}$) knee extension where lactate remained low, extracellular muscle water increased within 10 min from 0.32 to 0.48 l/kg dry wt and increased even further during the following 2 h to 0.64 l/kg dry wt, while total water content increased from 3.10 l/kg dry wt at rest to 3.60 l/kg dry wt at the end of exercise, which is a doubling of the interstitial water though only a 6% increase in intracellular water (590, 595). During intense (100% $V_{O_2\text{max}}$ for 6 min) knee extension, a relatively larger increase in intercellular water occurred from 2.78 to 3.14 l/kg dry wt corresponding to a 13% increase relating to large increases in muscle lactate concentration and thus osmotic pressure gradient (595). However, even in the latter condition, extracellular water doubled, and this increase may in both conditions relate to increased blood pressure as well as increased number of perfused capillaries which in combination may cause an increased filtration from the capillary bed to the interstitial space $V_v$. Interestingly, $V_v$ seems not to be altered by plasma osmolality (255). During static contractions ranging from 10 to 50% MVC, virtually no water fluxes occurred due to the high intramuscular pressures and low blood flow rates (554). Only at very low static contraction forces of around 5% MVC and if the contraction was sustained for as long as 1 h was an increase of total muscle water seen (from 3.03 to 3.30 l/kg dry wt) (591).

During whole body exercises such as bicycling, running, and swimming (45, 46, 122, 492, 506, 700), similar large translocations in total fluid are reported as during knee extension based on data of plasma water and total muscle water changes. With exercise dehydration, conflicting results are reported with one study showing increased muscle water content following 2 h at 50% $V_{O_2\text{max}}$ causing a 3% reduction in body weight (493), whereas another showed a decrease following 1.5 h at 70% $V_{O_2\text{max}}$ causing 2% reduction in body weight (122). The cause for this discrepancy is that in the latter case the muscle biopsy was obtained after exercise and a subsequent 30-min rest period in the supine position. The largest changes in total body water were reported after exercise and thermal dehydration resulting in body weight reduction of 4–6% (122, 123).

That fluid shifts affect ion concentrations was demonstrated by a series of experiments during which subjects performed repeated maximal exercise bouts of 1-min duration with 4 min rest between the bouts. $[K^+]_v$ increased with each bout as expected. However, $[Na^+]_v$ also increased with each exercise bout and fell back to almost normal level during rest (279). This was unexpected since $Na^+$ and $K^+$ were supposed to move in opposite directions across the muscle cell membrane. As revealed by the hematocrit, plasma volume decreased during the first and second bouts but was thereafter constant, indicating that plasma volume was only reduced initially and then remained constant despite the intermittent exercise. These data were interpreted by Sejersted et al. (573) in the following way. The initial uptake of fluid by the muscle cells leads to rapid decline of plasma volume, but because the cells take up only solute-free fluid, there will be an increase in the concentration of all plasma constituents. As soon as exercise ceases, intracellular CrP is rapidly resynthesized, and the fluid leaves the cells. However, the fluid will accumulate in the interstitium since the Starling forces that cause fluid uptake into the capillaries are small. However, the total extracellular volume is restored in the rest periods so that small solutes that pass the endothelium will be diluted. Thus $[Na^+]_v$ will be reduced toward normal again. Eventually, after all exercise bouts the plasma volume is also restored, but this process takes an hour or more. Thus the simultaneous increase in plasma concentrations of $Na^+$, $Cl^-$, and $K^+$ during high-intensity exercise is due to very different processes, and the fluid shifts may mask the uptake of $Na^+$ by the active muscle cells.

In conclusion, the largest changes in fluid volumes are seen in $V_v$ of the active muscle caused by hydrostatic forces as well as osmotic forces. During high-intensity exercise fluid is rapidly taken up by the muscle cells. A combination of exercise and dehydration that commonly occur will significantly modify this pattern, i.e., preventing increases in muscle water content. Fluid fluxes concurrent with electrolyte fluxes may markedly affect concentrations of the various ions and therefore seemingly mask the gross electrolyte shifts.

F. Recovery

At cessation of exercise, plasma $[K^+]$ as well as the $Na^+-K^+$ pump activity are elevated while $K^+$ being
translocated from active to remote tissues. The instantaneous termination of K⁺ release from contracting muscles due to the cessation of AP propagation implies that K⁺ is now taken up faster into Vₘ than it is released, something due to maintained high Na⁺-K⁺ pump rate. This will induce a decrease of [K⁺]v first in Vₛ and subsequently in the plasma and a regaining of lost K⁺ into the muscle. A rough estimate shows that the capacity of the Na⁺-K⁺ pump is sufficient to decrease plasma [K⁺] to resting level within seconds: if plasma [K⁺] at the end of exercise was maximal, i.e., ~10 mM and representing the [K⁺]ₑ of the total extracellular space Vₑ (15 l), then a maximally activated pump (~5.5 mmol·kg wet wt⁻¹·min⁻¹) in the entire muscle mass (40 kg wet wt) would clear [K⁺]ₑ to resting level within 20 s, and plasma [K⁺] would approach zero within 30 s. This illustrates that the problem following exercise is not how fast plasma [K⁺] can attain resting level but rather how recovery of K⁺ is regulated for plasma [K⁺] to remain within ranges compatible with bodily function. An important safety mechanism is the sensitivity of the Na⁺-K⁺ pump to [K⁺]ₑ. When [K⁺]ₑ falls below 4 mM, pump rate is rapidly reduced. Also, rapid decrease in [Na⁺]ₑ in the initial phase after cessation of muscle activity (184, 329), accentuated in the subsarcolemmal space (576), is associated with a decrease in Na⁺-K⁺ pump activity and plays an important role in downregulation of K⁺ clearance from the plasma (see sect. III). Nevertheless, [K⁺]ₑ, [K⁺]v, and [K⁺]v_mix show a fast decrease back to resting level within 2–5 min most often followed by an undershoot (246, 252, 372, 416, 442, 467, 658). This is reflected in a corresponding undershoot of [Na⁺]ₑ (329, 496) and subsequent slow recovery back to resting level over a 10- to 20-min period or more (61, 72, 442, 467).

The half-time for recovery of [K⁺]ₑ after exercise is clearly dependent on the muscle mass that was active. After intense running or bicycling engaging a large muscle mass, recovery of [K⁺]ₑ closely follows the rate of fall of [K⁺]ₑ since flow through the muscles constitutes a very large fraction of the total cardiac output, although [K⁺]v_mix also shows rapid and pronounced variations (442). The decay of [K⁺]ₑ or [K⁺]v_mix is almost monoeponential, with a half-time on the order of 25–100 s, which is independent of the previous exercise intensity, and which implies that it is also independent of how high [K⁺]ₑ was at end of exercise (442, 467). In other words, initial rate of fall of [K⁺]ₑ or [K⁺]v in this situation is proportional to [K⁺]ₑ at the end of exercise, which means that it is proportional to exercise intensity as noted by Vøllestad et al. (658). On this basis, the variations in [K⁺]ₑ with high-intensity running could be modeled by introducing a simple proportional regulator (467). Interestingly, the half-time for recovery of [K⁺]ₑ or [K⁺]v, is shorter for trained versus untrained as well as for sprint trained versus endurance trained (442, 467, 468). This is probably an effect both of larger muscle mass and increased number of Na⁺-K⁺ pumps (468).

In isolated muscles with no perfusion, the half-time of [K⁺]ₑ recovery was ~37 s (329). This is somewhat surprising since because of the small amount of available K⁺ in the interstitium one would expect a more rapid recovery. Interestingly, in the same study, the fibers were depolarized after the stimulation period, and the time constant for recovery of resting Eₘ was also 37 s, indicating that the recovery of [K⁺]ₑ is limiting for recovery of membrane function. It is important that investigators have made observations of membrane hyperpolarization after stimulation of perfused rat skeletal muscles (286), pointing to a larger contribution of the electrogenic Na⁺-K⁺ pump under these circumstances, as outlined in section II.A. The contribution of the Na⁺-K⁺ pump to the Eₘ may explain that in a study on the relationship between plasma [K⁺] and maximal knee-extension torque during 15 min of recovery after intense bicycle exercise the authors did not find a relationship between recovery of [K⁺]v_mix and torque (458).

After short-lasting exercise, the size of the undershoot of [K⁺]ₑ or [K⁺]v_mix seems to be linearly related to peak [K⁺]ₑ during exercise (442, 468) so that for a rise of [K⁺]ₑ of 1 mM the undershoot will amount to 0.12 mM. Both this relationship and the relationship between peak [K⁺]ₑ and initial reuptake rates may be fortuitous since K⁺ probably plays little or no direct role in the control of the Na⁺-K⁺ pump rate except when [K⁺]ₑ is lower than 4 mM. However, it is possible that the magnitude of perturbations of extracellular [K⁺] is proportional to the stimulus for Na⁺-K⁺ pump activation. Thus there seems to be a relationship between the actual rate of Na⁺-K⁺ pumping at cessation of exercise and the subsequent undershoot of [Na⁺]ₑ (496). Interestingly, β-adrenoceptor blocker completely abolished the postexercise undershoot of [K⁺]ₑ (246, 252). When exercise was performed during continuous administration of the β₂-agonist salbutamol, the exercise-induced activation of Na⁺-K⁺ pump rate was attenuated, and the undershoot was abolished (253). This indicates that catecholamines contribute to Na⁺-K⁺ pump activation during exercise.

[K⁺]v,a across the exercising muscle in the recovery period has been monitored by several investigators. Again, it should be emphasized that [K⁺]v,a only reflects K⁺ transfer across the cell membrane when [K⁺]ₑ is stable. When [K⁺]ₑ and simultaneously [K⁺]v,a are rapidly falling, the [K⁺]v,a may underestimate cell uptake rate of K⁺ since a large fraction is taken from the interstitial space Vₛ. In particular, immediately after exercise [K⁺]v,a falls almost instantaneously, reflecting uptake of K⁺ from the interstitium before [K⁺]v,a becomes negative (252, 658). One of the first studies reporting such data showed that following intense bicycling [K⁺]v,a was negative within 1 min after cessation of exercise and remained so.
for >10 min (391). After stepwise incremental bicycling to exhaustion, $[\text{K}^+]_{v-a}$ became negative already ~5–10 s after cessation of exercise and stayed so until the final sample at 3.5 min (252). After intense dynamic knee extension, blood samples obtained within 30 s showed negative $[\text{K}^+]_{v-a}$ values that remained so for more than 10–20 min (246, 332, 595). In the study by Juel et al. (332) of high-intensity exercise resulting in exhaustion within 3–4 min, ~70% of the total amount of 7.6 mmol K$^+$ released from the 2.7 kg active muscles was estimated to have accumulated in the plasma while the rest was taken up by other tissues, although not erythrocytes. During recovery, the kinetics of K$^+$ uptake were composed of a fast (<1 min) and a slow component. Within the first 30 s of recovery, the influx over the capillary membrane was 0.6 mmol/min and additionally an uptake of >1 mmol that had accumulated in the $V_v$ of the muscle was taken up into $V_c$. Based on various modes of calculations, the authors conclude that the pump was activated to accumulate ~2 mmol · min$^{-1}$ · kg wet wt$^{-1}$ at cessation of exercise and that altogether the K$^+$ that had accumulated into the plasma was taken up by the muscle in the initial phase (332).

This tallies with the slow recovery of muscle K$^+$ after repeated submaximal isometric contractions of the quadriceps muscle in humans (655). A total of 12 mmol K$^+$ was lost from the muscle throughout exercise that lasted for ~60 min. However, over the first 20 min of recovery, only 10% of the lost K$^+$ was reclaimed by the leg, and the continued uptake escaped detection.

Paradoxically, β-adrenergic receptor blockade not only causes a transiently increased $[\text{K}^+]_{v-a}$ after onset of exercise, but the negative $[\text{K}^+]_{v-a}$ after exercise is also transiently accentuated (246); this interpretation was made in light of the larger muscle K$^+$ loss during the exercise. At cessation of exercise, estimated Na$^+$–K$^+$ pump rates were the same in the presence and absence of blocker. However, because $[\text{Na}^+]_i$ was probably higher during β-adrenergic receptor blockade, it would take more time to reduce $[\text{Na}^+]_i$ and hence pump rates would remain higher for a longer period than in the control situation.

After static contractions, it is difficult to appropriately measure where K$^+$ moves. After a brief contraction, the elevated $[\text{K}^+]_i$ falls rapidly (295, 663). However, in the vein, $[\text{K}^+]_v$ transiently peaks since the reestablished flow will wash out accumulated K$^+$ from the interstitium (254, 295). With a more prolonged maximal contraction $[\text{K}^+]_v$ may rise slowly, indicating that K$^+$ leaks out to perfused regions or that flow is slowly reestablished. The consequence is that there is only a small or no peak of $[\text{K}^+]_v$ when contraction ceases (189, 254). Hence, it is not surprising that appearance of a negative $[\text{K}^+]_{v-a}$ seems somewhat delayed (189, 322, 554, 591, 602). Interestingly, when the same tension-time integral was performed intermittently (5-s contraction, 5-s relaxation) and compared with sustained static, much larger negative $[\text{K}^+]_{v-a}$ values were seen in the recovery despite similar positive $[\text{K}^+]_{v-a}$ values during the contractions. One reason for this finding may be the larger K$^+$ loss during intermittent contractions caused by high blood flow in the relaxation periods (602). Furthermore, when various contraction forces were sustained until exhaustion, 1 h elapsed before K$^+$ balance was restored after the highest contraction level of 40% MVC sustained for 2.7 min. This implies that the more prolonged the exertions, the longer the K$^+$ recovery time.

When blood flow was occluded immediately before cessation of a 3-min static forearm contraction, an uptake of K$^+$ from the extracellular space in the muscle occurred. This could be anticipated from the comparative differences between the decline of the $[\text{K}^+]_i$, in the effluent blood at reperfusion after 3 min and the nonoccluded condition (189, 322). The K$^+$ loss during 3 min of 15 and 30% MVC were ~0.2 and 0.5 mmol, respectively. During occlusion, 0.05 and 0.14 mmol were taken up, respectively, which means that ~25% of the K$^+$ loss must additionally have been released from the $V_c$ of the contracting muscles and was available in the intramuscular $V_v$ for immediate uptake. This also implies that at the end of contraction fairly high $[\text{K}^+]_a$ values were attained, which were calculated to be on the order of 1 mM above $[\text{K}^+]_i$, following 3 min at 30% MVC. After 7.5 min, less than half the K$^+$ loss had been taken up again (322).

Various manipulations have been imposed to affect the recovery of muscle K$^+$. Intake of large amounts of K$^+$ in the fluid intake for rehydration increasing plasma $[\text{K}^+]$ to 5.4 mM over a period of 2 h did not affect muscle K$^+$ uptake (493). Thus it seems that the availability of K$^+$ in the plasma does not limit the reuptake over any prolonged period of time. On the other hand, prolonged low-intensity contractions result in an equally prolonged recovery phase, which may last for more than 1 h (76). Manipulation of plasma pH, lactate, or HCO$_3^-$ had no effects on the time history of recovery (72).

In conclusion, recovery of K$^+$ balance after exercise is composed of an initial fast component, which may be mainly dependent on the availability of K$^+$ in the $V_v$ of the muscle as well as the Na$^+$–K$^+$ pump activity at cessation of exercise. There is a second slow component, which we know little about, but one can speculate that lack of intracellular Na$^+$ for pump stimulation may well be the reason for very slow restoration of muscle K$^+$ content. Also, K$^+$ that has been taken up by remote tissues may be released very slowly and transferred back to the previously active muscle. The durations of the fast and slow components depend on the intensity, mode, and duration of the preceding exercise relative to the state of training of the muscle. The mechanisms of the tightly controlled, time-dependent regulation of the Na$^+$–K$^+$ pump during recovery remain largely unknown, but in the intact organism, high priority is given to maintain plasma $[\text{K}^+]$ at a
level compatible with normal bodily function rather than to maximal uptake rate in the exercising muscles.

**IX. POTASSIUM AND FATIGUE**

**A. Definition of Fatigue, Exhaustion, and Contractile Failure**

Over the last 100 years since Mosso (479) first studied fatigue systematically, a number of different definitions of exercise-induced fatigue in skeletal muscle have been presented. According to a rather general definition, fatigue is the transient loss of force-generating capacity resulting from preceding physical activity (23, 587). In line with this, the vast majority of studies report a decline in isometric tetanic tension or static MVC as an expression of fatigue. These variables, however, only describe changes in the one dimension: force, while the mechanical performance of muscle also includes the dimensions time and distance. These are required for quantification of the variables: rate of force rise and decay, shortening velocity, work (= force \( \times \) distance), and power (= force \( \times \) velocity). Changes of the latter compound variables, and not only force, are important for quantifying changes in the capacity of the muscles to perform a movement (e.g., locomotion when moving body segments) as well as the capacity to sustain external forces acting upon the body.

Following muscle activity, peak force is reduced along with relaxation rate during tetanic as well as twitch force development. This reveals a decrease in cross-bridge force (less force per cross bridge and/or fewer attached cross bridges) as well as in cross-bridge turnover rate. Following high-intensity muscle activity, both force and shortening velocity decline, although the latter may not decline much following prolonged exercise and normally not until tetanic force is decreased by at least 10% (168, 203). The general finding is that tetanic force declines more than velocity, and muscle power is compromised more than either of these two (140, 202, 388, 689). Furthermore, the magnitude of force or velocity decline depends on the pattern and duration of the preceding activity.

The interrelationship between the mechanical variables and characteristic changes with fatigue described above are shown in Figure 10. Fatigue has been defined as a failure to maintain a required force or exercise intensity (169); however, this condition should rather be referred to as the end point of fatigue or exhaustion. The gradual decrease in mechanical performance or deteriorated function that occurs from the beginning of muscle activity and that is an integral part of physical activity is here defined as fatigue, in line with previous suggestions (51, 659). Contractile failure refers to the total inhibition of force development and as such is beyond the point of exhaustion where a preset kinetic performance can no longer be sustained.

In many reviews, the possible steps involved in the fatigue process have been specified as excitation of motor cortex centers, excitatory drive to motor neurons, motor neuron excitability, neuromuscular transmission, sarclemma AP transmission, t-tubular AP transmission, Ca\(^{2+}\) release from the sarcoplasmic reticulum, cross-bridge cycling, and energy turnover rate at various sites (202, 565, 592, 691). In particular, distinctions are made between central and peripheral fatigue (53, 54). The latter relates to failure of processes at or distally to the neuromuscular junction. Close associations between voluntary force development and direct electrical stimulation of force suggest that central fatigue represents only a minor factor. For instance, after high-intensity isokinetic or intermittent static knee extension, central fatigue accounted for <10% of the decrease in muscle power and force, respectively (39, 53, 318). Interestingly, even in the recovery phase, voluntary and electrically elicited force development are closely linked (39). However, without wanting to dismiss the considerable research that has been carried out in the area of central fatigue including the significance ofafferent activity (223), the present review on fatigue will only deal with peripheral fatigue and focus on the
processes at the muscle surface membrane that are affected by changes in $[K^+]_s$ as well as $[K^+]_c$. Fundamental differences exist in the kinetics of the mechanical activation of skeletal and heart muscle (167), which also are not detailed here.

There still seems to be some confusion with respect to the terminology of low-frequency fatigue versus high-frequency fatigue. Originally, the concept of low-frequency fatigue was introduced to describe the specific loss of force during a test stimulation at low frequency in the recovery period after fatiguing exercise (170). Although the muscles could still be nearly fully activated at high stimulation frequencies, a large impairment of force development had occurred at low stimulation frequencies, and this state was referred to as low-frequency fatigue (LFF). The main features of LFF are as follows: 1) relative force loss at low frequencies of stimulation is greater than at high frequencies, 2) recovery is slow, and 3) LFF persists in the absence of gross metabolic or electrical disturbances (326, 660). It should be pointed out that this type of fatigue can be caused by a variety of activities and is not particularly caused by activities of low firing rates such as low-intensity contractions or low-frequency stimulation, but it is characterized by large force losses when tested at low frequencies of stimulation. The most important feature of LFF is that it is long lasting (hours or days), and “long-lasting fatigue” would be an alternative choice of terminology. The recovery from LFF is probably related to protein turnover rates necessary for regeneration and repair of altered or damaged proteins in the muscle (70, 325, 328) rather than to the recovery of metabolic and electrolyte balances. The site of LFF may be related to the mechanisms of $Ca^{2+}$ release (510, 687, 702), and the understanding of this phenomenon is important for prevention of muscle disorders such as occupational myalgia (659). However, it is most likely unrelated to $K^+$ balance.

High-frequency fatigue (HFF) is the preferential loss of force at high as opposed to low frequencies of stimulation. HFF is characterized by the following: 1) loss of force during high-stimulation frequencies and is rapidly reversed by decreasing the frequency of stimulation, 2) loss of force is accompanied by a decrease in amplitude and duration of the surface membrane recorded AP, and 3) loss of force is exacerbated if $[Na^+]_s$ is decreased or $[K^+]_s$ is increased (326). Several authors use the term HFF synonymously with the reduced force seen during protocols of high-frequency stimulation used in studies of metabolic and electrolyte perturbations and do not distinguish between the fatiguing exercise and a test protocol used to characterize the fatigue (79, 81, 386, 691). Rather than using phrases such as “fatigue from high- and low-frequency muscle stimulation” (470), although they are correct, terms such as “fatigue induced by high-intensity contraction” and “fatigue induced by low-intensity contraction” would be more advisable to avoid the misunderstanding of a direct coupling between contraction mode and fatigue characteristics. The characteristic of fatigue induced by different contraction modes may be specified as, e.g., HFF, LFF, a combination of these two, or even other types of fatigue characteristics yet to be specified.

**B. Intracellular/Extracellular $[K^+]$ and Contractility**

The sarcolemma and the t-tubular system are frequently proposed sites for fatigue development thought to be causally related to changed $[K^+]_s$ gradients (202, 460, 467, 569, 592). The magnitude of these changes depends on the contraction mode, intensity, and duration as presented in section VIII. The mechanical performance of the muscle in relation to $[K^+]_s$ in the various compartments during in vitro manipulations of electrolyte concentrations in nonfatigued and stimulated muscle preparations as well as in human voluntary exercise is discussed below.

1. Effects of extracellular ion perturbations on contractile properties

The effects of changes in $[K^+]_s$ on electrical properties as described in section IV are complex, and there is not a simple linear relationship between $[K^+]_s$ and force development. Moderate increases may even cause force potentiation, but at high $[K^+]_s$ (i.e., higher than 8–10 mM), force development is suppressed (20, 60, 80, 101, 112, 331, 386, 536), and again at even higher $[K^+]_s$ contracture develops (161). These observations may question the hypothesis that $K^+$ is in fact a major determinant for normal muscle fatigue since these high $[K^+]_s$ are seldom thought to occur during exercise. However, $[K^+]_s$ should not be seen in isolation, since a number of simultaneous changes occur in the in vivo condition that may assist $[K^+]_s$ to depress force.

First of all, increases in $[K^+]_s$ develop in combination with decreases in $[K^+]_c$ (329, 414, 599), which may depolarize the sarcolemma beyond −60 mV (329–331, 386, 414). At this $E_m$ a large reduction in tetanic force has been reported in rat muscle (80, 414) as well as amphibian muscle (536) while force potentiation may occur in the latter muscle during twitch contraction (386). In a detailed study on mammalian muscle (mouse), the relationship between $[K^+]_c$, $E_m$, and contractility demonstrated a biphasic effect: when $[K^+]_s$ had depolarized the $E_m$ to less than −60 mV, the force decline was limited (<20%) and probably related to an altered AP profile (phase 1), but when the $E_m$ had decreased to a range of between −60 and −55 mV, a marked force decline was seen, probably...
related to inexcitable fibers (81). Phase 1 may be considered as an $E_m$ safety margin, and the lack of a linear relationship between electrical and mechanical events does not exclude causality.

Second, during activity, simultaneous Na$^+$ translocations occur in opposite directions to K$^+$, and it has been proposed that neither K$^+$ nor Na$^+$ alone but rather the reduced ion gradients in combination can be considered as an important factor for force decrease (60). Both mouse and rat soleus muscles were exposed to various combinations of increased $[K^+]_i$ and reduced $[Na^+]_i$ in the bathing solution, each of which showed little effect on twitch and tetanic force, whereas in combination they were clearly synergistic in inducing a major impairment in tetanic force (82, 511) and a parallel attenuation of the M-wave area (512). Interestingly, tetanic force loss induced by elevated $[K^+]_i$ (8 mM) and lowered $[Na^+]_i$ (100 mM) was partially reversed with high $[Ca^{2+}]_i$ (10 mM) and markedly exacerbated with low $[Ca^{2+}]_i$. Similarly, high $[Ca^{2+}]_i$ was seen to reduce while low $[Ca^{2+}]_i$ was seen to increase the fatigue that occurred with repeated intermittent tetanic stimulation (82).

Third, Na$^+$-K$^+$ pump activity may conceal pure effects of changed $[K^+]_i$ and $[Na^+]_i$ gradients because these ions per se will activate the Na$^+$-K$^+$ pump, which via its electrogenic effect will influence the $E_m$ (see sect. iv). Thus, when the activity of the pump was partially inhibited by ouabain, the decrease in force development induced by high $[K^+]_i$ (10–15 mM) was accelerated although later decreased again when the pump was stimulated (104, 369). Also, in line with the above studies, a decrease in $[Na^+]_i$ to 25 mM decreased tetanic force to 30%, and this was considerably accentuated by increasing $[K^+]_i$ (511). However, stimulation of the Na$^+$-K$^+$ pump by, e.g., catecholamines, does lead to decreases in $[Na^+]_i$, hyperpolarization, and rapid recovery of force and M-wave area (511, 512). In the in vivo condition, catecholamines increase with exercise and may contribute to an attenuation of the K$^+$-dependent force decrease. In contrast, reduction in the Na$^+$-K$^+$ pump activity markedly decreases contractile endurance during stimulation at high frequency (495). In short, the increased Na$^+$-K$^+$ pump activity will attenuate the reduction in force-developing capacity in muscle fibers exposed to reduced $[K^+]_i$ and $[Na^+]_i$ gradients across the sarcolemma (453, 512).

Last, but not least, the muscle membrane includes surface sarcolemma as well as t tubule, the latter previously pointed out as being a possible site for fatigue (690). It is consistently argued that changes in the concentration of electrolytes in the t tubule may be much more pronounced than in the interstitium as discussed in section iv, although it is at present unclear to what extent these changes affect t-tubular function.

## 2. Relationships between activity-induced ion perturbations and muscle function in animals

A key question is whether the activity-related changes of $[K^+]_i$, $[K^+]_e$, $[Na^+]_i$, and $[Na^+]_e$, and the associated effects on excitability, AP amplitude and duration, and conduction velocity can be causally related as proposed and discussed in section ivC. Clearly, such changes are associated with reduced rate of force development (10-Hz stimulation) as well as peak force amplitude (20- to 50-Hz trains) (259, 286, 330, 369). The activity-induced changes in force as well as AP are generally more profound in fast-twitch than in slow-twitch fibers (180, 259, 330).

From the foregoing it is necessary to distinguish between fatigue induced by high-intensity contraction and fatigue induced by low-intensity contraction. A dissociation of time courses for the changes in AP and force reduction may clearly occur, showing that under some conditions (40 Hz 0.33-s trains every second for 360 s) the main cause of fatigue is unrelated to the electrical events (180) and that a component of LFF is present. On the other hand, in another study the magnitude of force loss and recovery time were related to the magnitude of K$^+$ flux as manipulated by different HCO$_3^-$ levels (441). Also, caution must be taken when relating only changes in AP shape to a reduction in force amplitude at a given stimulation frequency, because the sarcolemma AP frequency may be much lower than the stimulation frequency following fatiguing contractions (27). However, a concomitant leftward shift of the force-frequency curve (635) may in part offset the effect of an inefficient triggering of AP.

Different modes of fatiguing stimulation have been studied in single frog fibers showing that the changes in force, $E_m$, and AP in the contraction as well as recovery periods are markedly different (386, 387, 688). It is the development of high $[K^+]_i$ that occurs during intense contractions and the ensuing depolarization of muscle fibers that may interfere with excitability and thus contractility of muscles as discussed elsewhere and as reviewed by Fitts (201). Recently, Overgaard et al. (512) noted a close correlation between the M-wave area and force during fatiguing stimulation of the isolated rat soleus muscle. $[K^+]_e$ and $[Na^+]_e$ recover rapidly and with a time course that shows a correlation with the fast phase of recovery (26, 635), and in line with this the M wave and force also recover in parallel (512). This emphasizes that the changes at the sarcolemma are of significance mainly with intense activity, there is a safety margin before their changes have an effect on tension development, and these ion perturbations are quickly normalized. Thus the membrane theory (i.e., impaired membrane excitability) cannot explain the slow recovery phase.
3. Exercise-induced changes in electrolyte, electrical, and mechanical events in human muscle

Evidence of changes in the electrical events with fatigue in humans is seen from surface electromyography, M-wave, and motor unit AP recordings, since the glass electrodes for intracellular AP recordings are not applicable during human muscle contraction. The characteristic changes in the surface electromyogram are increased root mean square amplitude and decreased frequency of the power spectrum of the signal (55, 337). The magnitude of this shift relates to endurance time and was therefore termed a “fatigue index” (423). A detailed study (55) on the underlying changes in the AP shape, conduction velocity, and firing frequency revealed important differences depending on contraction intensity. At 20-Hz stimulation there was little change in AP amplitude, but there was an overall prolongation of the waveform. At 50 Hz this prolongation was more marked, and a decrease in AP amplitude occurred. At 80 Hz, the same changes were seen, but in addition, a failure to initiate AP was seen. The increases in RMS amplitude were for any frequency directly proportional to changes in the AP area. Little change was also seen in AP amplitude during sustained maximum voluntary contraction, although total AP area generally increased as did conduction time, although this could not fully account for the power spectral changes (52). A consistent finding during intense voluntary contraction is a decrease in firing rate of single motor units (327). With submaximal contractions the electrical events at the sarcolemma seem not to become deteriorated since the M wave is well maintained (53) or even potentiated (285, 686).

Muscle fatigue studies in humans have for many years focused on metabolism as a limiting factor with respect to the availability of substrates and accumulation of metabolites (278, 280). However, lack of ATP was never documented since the [ATP] with voluntary contractions never approached zero, which would cause rigor and cell death (201, 701). Interestingly, Bigland-Ritchie and co-workers (55) pointed to [K+] in as being causally related to the changed electrical events as well as mechanical responses. Controlled tests in humans quantifying the decrease in mechanical muscle performance with activity (e.g., MVC or electrical stimulation at high and low frequencies) in combination with electrolyte changes in the muscle are rare (572, 591, 661), and more often studies have been conducted analyzing the electrolyte changes at exhaustion, i.e., at the end point of fatigue, where the preset exercise load could no longer be maintained.

After 3 min of 30% MVC, the M wave tended to be potentiated while [K+] in was elevated in the early phase of recovery (686), which led the authors to conclude that excitability was maintained despite increased extracellular [K+]. Here it is crucial to make a distinction between [K+] in and [K+] os which may have quite different time histories. To estimate the amount of K+ accumulated in the interstitial space of the muscle, recovery after 30% MVC was studied both with and without occlusion of the circulation (189). Without occlusion a pronounced peak in [K+] os was seen when contraction ceased (Fig. 1). When the circulation was arrested for the first 3 min of the recovery period, no peak appeared when occlusion was released. Thus there must have been a K+ uptake by the muscle cells. This reuptake was estimated from [K+] os and blood flow and indicated that [K+] os was at least 1 mM above measured [K+] os at cessation of contraction. However, in the early phase of recovery, K+ located in the interstitium is taken up by the cells, and there may be a delay before this can be detected at the venous sampling site. Taken together, this implies that there may be a dissociation between [K+] os and [K+] in due to the time required for capillary blood to reach the sampling site. This means that [K+] in may not have mirrored the [K+] gradient across the sarcolemma when the M wave was elicited (686). Interestingly, evidence of LFF was presented during 25% MVC sustained until exhaustion either as a continuous contraction or with different intermittent protocols, and LFF persisted for 4 h after all exercises but was fully recovered after 24 h (77). In conjunction with LFF, an increased [K+] in of ~1 mM during the contraction was also seen while a large uptake of K+ occurred throughout the first 60 min of recovery. The recovery of K+ following sustained contractions of 10, 25, and 40% MVC until exhaustion was dependent on the contraction intensity, and after 1-h recovery, the largest deficit remained after a 10% MVC, whereas full recovery had occurred after a 40% MVC (76). More recently, in studies of intense knee extension to exhaustion, it was concluded that fatigue was not caused by elevated acidity per se (28) but suggested that it may be related to increased [K+] in (31). In line with this a study, comparing fatigue induced with contractions of identical tension-time products (150% MVC · min) but different durations and duty cycles, performed at forces of 10, 25, and 40% MVC either continuously or intermittently, showed that only following the low mean contraction intensities (implying longer contraction times) was K+ balance still not regained after 1-h recovery (75). However, this study demonstrated LFF in all experiments after 1-h recovery in terms of a 15–20% decreased force response at 20-Hz electrical stimulation while force at 100 Hz was unchanged. This is in line with the statement presented previously that K+ deficit may not play a major role in LFF. Interestingly, MVC had recovered fully after 1 h following one of the high-intensity, short-duration contractions but not the others, supporting the idea that K+ balance is decisive in high-intensity contraction. When 5% MVC was sustained for 1 h, ~5% of total muscle K+ was lost and MVC had decreased.
substantial loss of $K^+$ causes rapid and pronounced increase of $[K^+]_e$, e.g., in occupational settings. This condition is considered a risk factor of the muscle without being recognized by the central nervous system. This implies that major intracellular changes may occur during low-intensity exercise being primarily located at muscle surface membrane and being rapidly reversible. In contrast, with lower intensity of activation and sustained for prolonged duration, the sites of fatigue are increasingly to be identified beyond the muscle membrane (i.e., in the extracellular space). This has important consequences regarding the feedback from the contracting muscle for fatigue perception, since the sensory afferent nerves originate in the interstitial space of the muscle and thus cannot respond to intracellular changes. This implies that major intracellular changes may occur during low-intensity prolonged contractions that may induce degeneration of the muscle without being recognized by the central nervous system. This condition is considered a risk factor in the development of muscle disorders that must be recognized in the implementation of preventive strategies, e.g., in occupational settings.

C. Conclusions

We conclude that with high-intensity exercise that causes rapid and pronounced increase of $[K^+]_a$ and a substantial loss of $K^+$ from the active muscle, it is quite likely that the electrical consequences of the ions shifts across the muscle surface membrane are primary causes of fatigue that recover quickly and relate to HFF. Also, LFF develops during high-intensity exercise. However, the relative role of LFF becomes gradually more important with lower exercise intensities, and the slow recovery of LFF involves intracellular perturbations of the $Ca^{2+}$ homeostasis.

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