Distribution of extracellular potassium and its relation to electrophysiologic changes during acute myocardial ischemia in the isolated perfused porcine heart

RUBEN CORONEL, M.D., JAN W. T. FIOLET, PH.D., FRANCJEN J. G. WILMS-SCHOPMAN, ALEXANDER F. M. SCHAAPHERDER, M.D., TIMOTHY A. JOHNSON, PH.D., LEONARD S. GETTES, M.D., AND MICHEL J. JANSE, M.D.

ABSTRACT An experimental approach is described to quantitate inhomogeneity in extracellular K concentration ([K⁺]₁₀_u) in the presence of ischemia and to relate this inhomogeneity to the electrophysiologic changes. Extracellular potassium concentration and local direct-current electrogrograms from the same sites were measured in isolated perfused pig hearts with the use of multiple electrodes. Dispersion of [K⁺]₁₀_u is described under three conditions: (1) during regional ischemia in the "central zone" and the "borderzone," (2) during global ischemia, and (3) during perfusion of the heart with a high-K perfusate. Inhomogeneity was greatest during regional ischemia, especially in the borderzone, where generally lower concentrations were measured. When during regional ischemia the normal zone was perfused with a high-K perfusate, dispersion in the ischemic borderzone diminished, and higher concentrations than in the central zone were measured. During global ischemia inhomogeneity was slightly larger than during high-K perfusion. Dispersion during the latter was considered due to experimental error. A decrease in [K⁺]₁₀_u during regional ischemia after the initial increase was closely correlated with electrical recovery of the electrograms. This decrease occurred earlier in the borderzone than in the central zone. During ischemia [K⁺]₁₀_u was not related to the occurrence of monophasic electrograms, which are indicative of the absence of local regenerative responses. For every single electrode position a linear relationship between TQ depression and [K⁺]₁₀_u was found, the slope of which varied with the position of the electrode. When all sites were taken together, there was no correlation between TQ depression and [K⁺]₁₀_u. We conclude that: (1) inhomogeneity of K⁺ is largest in the borderzone, (2) potassium flows from the ischemic zone into the normal zone, (3) transient electrical recovery is related to a decrease (after an initial increase) in [K⁺]₁₀_u, which is at least partly due to a flow of K⁺ toward the normal zone, (4) monophasic ("block") electrograms can be recorded from intrinsically excitable tissue, (5) for every single site in the ischemic region there is a linear relationship between local [K⁺]₁₀_u and local TQ segment depression, and (6) the degree of TQ depression at a particular site is not a reliable index of the degree of ischemic injury at that site.


THE EARLY PHASE of myocardial ischemia is associated with the occurrence of lethal ventricular tachyarrhythmias. Harris et al.¹ were among the first to identify an increased extracellular potassium concentration ([K⁺]₁₀_u) as a major determinant of these arrhythmias by measuring the potassium concentration in blood samples drawn from the coronary veins draining the ischemic region. The importance of the rise in [K⁺]₁₀_u with respect to electrophysiologic changes during ischemia was further demonstrated in experiments in which these changes could be simulated by perfusing a heart with a Tyrode solution containing a high potassium concentration in combination with a low pH and hypoxia.² Direct measurement of [K⁺]₁₀_u has become possible since the introduction of potassium-sensitive electrodes.³ A characteristic biphasic time course of extracellular potassium accumulation has been de-
scribed during myocardial ischemia: an initial fast rise is followed by a plateau, during which a transient decrease in $[K^+]_{\text{out}}$ may occur, until a second phase of increase in $[K^+]_{\text{out}}$ heralds the onset of irreversible injury.\textsuperscript{4, 5} Apart from these time-dependent changes in $[K^+]_{\text{out}}$, regional differences were observed by Hill and Gettes\textsuperscript{5} in an open-chest porcine preparation. The dispersion of potassium accumulation resembles the inhomogeneity encountered in other variables during regional myocardial ischemia, such as refractory period, resting membrane potential, excitability, conduction times, and TQ segment changes.\textsuperscript{6-9} Inhomogeneity of electrophysiologic variables is thought to favor the occurrence of reentrant arrhythmias.\textsuperscript{10-12}

This study was undertaken to quantify the inhomogeneity of extracellular potassium accumulation during regional and global myocardial ischemia and to relate the differences in potassium concentration to differences in electrophysiologic variables in a preparation with poor collateral vessels. For this purpose we measured $[K^+]_{\text{out}}$ with multiple potassium-sensitive electrodes in the pig heart.\textsuperscript{13} Additionally, the mechanism underlying the dispersion of extracellular potassium accumulation was investigated.

It appears that in regional ischemia a true borderzone for $[K^+]_{\text{out}}$ of about 1 cm width exists, with intermediate values and a large degree of dispersion. The reason for the existence of such a $K^+$ borderzone is that potassium diffuses toward the normal myocardium.

**Methods**

Sixteen pigs weighing 15 to 20 kg were anesthetized by intravenous injection of sodium barbital (20 mg/kg). After tracheal intubation, respiration was artificially maintained and a midsternal thoracotomy was performed. Heparin (1000 U) was administered intravenously and while 1 liter of modified Tyrode’s solution (containing 136 mM Na\textsuperscript{+}, 4.7 mM K\textsuperscript{+}, 1.5 mM Ca\textsuperscript{2+}, 0.7 mM Mg\textsuperscript{2+}, 0.5 mM PO\textsubscript{4}\textsuperscript{3-}, 137 mM Cl\textsuperscript{-}, 28 mM HCO\textsubscript{3}\textsuperscript{-}, and 20 mM glucose) was infused through a superficial femoral vein, blood was collected from the anterior caval vein. This procedure yielded approximately 2 liters of a blood-Tyrode’s mixture to be used as perfusion fluid. The heart was made to fibrillate by application of a direct current, it was dissected free, and as quickly as possible, submerged in a Tyrode solution at 4°C. The aorta was cannulated above the aortic valves and the preparation was mounted on a Langendorff perfusion setup and defibrillated by a direct-current countershock. The left anterior descending (LAD) artery was cannulated and connected to the perfusion system separately. A ligature was passed under the circumflex (CX) artery.

**Perfusion system.** Perfusion was carried out with two recirculatory systems, either of which could be directed to the heart by a three-way stopcock placed immediately above the aortic cannula. In one of the recirculatory systems a normal potassium concentration was maintained (4.8 ± 0.5 mM, mean ± SD), while in the other $[K^+]$ was raised by addition of KCl to reach values of 15 to 20 mM, which would allow defibrillation of the heart when necessary (this was preferred to defibrillation by countershock, which could damage the buffer amplifiers once the electrodes were inserted). The blood-Tyrode’s mixture flowing out of the coronary sinus was collected in a funnel placed underneath the heart. From the funnel the blood flowed back to the recirculating system. Switching between the systems resulted in minor changes in the potassium concentrations of the two types of perfusion fluid caused by some mixing between the two circulations. The LAD artery alone could be selectively perfused by either circulatory system. Switching between the systems took less than a second. Both systems could be regulated to maintain constant flow (ranging between 80 and 120 ml/min under control conditions) or constant pressure (ranging between 40 and 50 mm Hg). Perfusion solutions were gassed with a mixture of room air and CO\textsubscript{2}, adjusted to a pH of 7.35 to 7.45 in the perfusion fluid, and subsequently passed through a heat exchanger. The temperature of the heart was 37°C; during regional ischemia it fell at most by 2°C in the ischemic area. No provisions were present for keeping myocardial temperature constant during global ischemia: in the midmyocardium temperature fell at most by 5°C over 10 min of global ischemia.

**Stimulation.** The heart was paced through a stimulating electrode pair placed on the right ventricular outflow tract with the use of rectangular current pulses of 2 msec duration. The basic cycle length was between 350 and 450 msec in the various experiments and was kept constant throughout an experiment.

**Measurement of extracellular potassium activity.** Extracellular potassium activity was measured with flexible potassium-sensitive electrodes with valinomycin, embedded in a polyvinyl chloride (PVC) matrix, as the ion-selective ligand. Each electrode consisted of a potassium-sensitive terminal and a reference (Ag-AgCl) terminal. The electrodes were constructed as follows: Two 40 cm long, electrically insulated silver wires (diameter 0.2 mm) were glued together at one end, in a parallel side-to-side fashion, with a drop of cyanoacrylate glue (Loctite), in such a way that there was no electrical contact between the two cores. At a distance of 10 cm from the common end the insulation of both wires was locally removed over a length of 0.5 mm. At this point the bare silver was bleached by sodium hypochlorite, resulting in an AgCl deposit. A small droplet of a solution of titanium dioxide in trichloroethane was deposited on the AgCl layer both for mechanical protection and to serve as an internal reservoir of potassium for the potassium-sensitive terminals. The latter was obtained by soaking the electrodes for 10 min in an isotonic, 10 mM KCl solution. Subsequently, the electrode terminals were immersed in a solution of the potassium-sensitive membrane in tetrahydrofuran. The composition of the membrane was: 2 wt% valinomycin, 29 wt% PVC, and 69 wt% dioctyl sebacic acid. The membrane of the reference electrode terminal was punctured with a fine needle. Finally, a 3-0 suture with anatraumatic straight needle (Ethicon) was glued to the common proximal end of the electrode pair, and a mark was painted on the wires 5 mm distal to the electrode terminals.

Response time of the electrodes was well under 1 sec for a tenfold change in potassium concentration. The electrodes were insensitive to changes in protein content and osmolality.

Up to 64 electrode pairs were positioned in the left ventricular wall by inserting the needles perpendicularly into the epicardial surface. When the needle emerged from the cavity or the posterior wall gentle traction was applied to align the painted depth mark on the wires with the epicardial surface. The total number of electrode pairs inserted in 16 hearts was 639. The distance between the electrodes was 4 to 10 mm. The majority of the electrodes was positioned in the part of the ventricle supplied by the LAD artery, the remainder in that supplied by the CX artery.
The electrodes were connected to high-input impedance (>10^13 Ω) low-bias current (<1 pA) preamplifiers (Burr Brown, OPA111). Differential signals from one electrode pair were direct-current amplified, fed to an analog-to-digital converter (1 sample/4 msec), and written into a circular memory buffer in a PDP 11/34 computer. This buffer contained the most recent 2 sec of data from all signals. At any desired moment the content of the buffer could be stored on disk for later analysis. In the same manner electrograms from the reference electrodes were direct-current amplified against a common nonpolarizable reference electrode attached to the aortic root and processed identically. Selected electrograms and K signals were monitored on line on an Elema 16-channel inkwriter and on a low-speed chart recorder, respectively.

Before and after each experiment K electrodes were calibrated at room temperature in two isotonic solutions with a [K+] of 1 and 10 mM, respectively. In addition, the heart was perfused with a high-K+ solution for several minutes as an in situ test of the electrodes. Data obtained from the electrodes were accepted only when all of the following requirements were met: (1) both calibrations produced a 52 to 58 mV change per tenfold change in potassium concentration, (2) the electrogram recorded from the reference electrode showed less than 2 mV ST elevation under control conditions, (3) the potassium trace showed less than 15 mV/hr baseline drift, (4) the electrode response to perfusion with a high [K+]—containing solution (test perfusion) was complete within 2 min and agreed with the calibration in vitro within experimental error, and (5) the reference electrode gave a stable recording. By strict application of these criteria the data from only 317 electrodes (49%) were accepted: 171 electrodes were rejected because of ST elevation, 114 for a bad response to test perfusion, 33 had an unstable reference electrode, and four displayed too much baseline drift. The electrodes that were accepted had a mean Nernst-constant at room temperature of 56.0 ± 1.6 mV per decade (n = 317). Recordings from the potassium electrodes were corrected for baseline drift by fitting a straight line between two readings with identical potassium concentrations. For this purpose and to determine the starting level of [K+] before each intervention, samples from the perfusion fluid were drawn at regularly spaced intervals and [K+] was determined by flame photometry.

Baseline drift was maximal and in positive direction after insertion of the electrodes; it decreased progressively in time until after 1 to 2 hr it measured approximately 1 mV/hr.

Experimental protocol. After the electrodes had been inserted the heart was allowed to recover for 1 hr. Recovery time of 1 hr allowed the heart to function under conditions similar to those during normal perfusion, and recovery from fibrillation was adequate.

In seven hearts 7 to 10 min periods of regional ischemia were performed, followed by global ischemia (five hearts) and test perfusion (six hearts). Sixty-seven electrode pairs were involved in the periods of global ischemia; 114 electrode pairs were situated in the ischemic zones during regional ischemia. In five hearts short periods of regional ischemia were combined with high-K+ perfusion of the remainder of the heart.

In four hearts long (15 to 20 min) periods of regional ischemia were induced. In these experiments 61 electrode pairs were situated in the ischemic LAD zones.

Definitions. In this study the following definitions were used:

The ischemic zone is the area in which potassium accumulates extracellularly after clamping a coronary artery.

The normal zone is the area in which the extracellular potassium concentration remains unchanged during regional ischemia.

The border is the line separating the normal zone from the ischemic zone.

The borderzone is an area extending 10 mm from the border into the ischemic zone.

The central zone is the ischemic zone with the exclusion of the borderzone.

Presentation of data. Data derived from the potassium electrodes are presented as the change in the membrane potential (∆EK, in mV) normalized for electrodes with a calibration factor of 60 mV per decade of change in potassium concentration. This was preferred over [K+] as a variable because an error in the determination of the calibration factor or the drift correction line has a proportional effect on ∆EK and a nonlinear effect on [K+]out. Moreover, since extracellular K+ is the major determinant of the resting membrane potential, ∆EK can directly be interpreted as the local change in the resting membrane potential.

Means are given with SDs unless stated otherwise. Statistical significance was tested with a double-sided t test, or when appropriate by comparing 95% confidence intervals.

Results

Figure 1 shows typical tracings from three potassium-sensitive electrodes during three different subsequent interventions. Panel a presents the response of the electrodes to a global test perfusion with a [K+] of 8.1 mM. Note that none of the electrodes recorded a return to the control value; this was caused by spilling of some potassium from the high-K+ perfusate into the normal-K+ solution. This was confirmed by flame photometry of samples taken from the normal-K solution. Panel b shows the response of the same electrodes during a 10 min period of global ischemia. The first rapid phase of potassium accumulation and the subsequent plateau phase are clearly seen. In this particular experiment ventricular fibrillation (VF) occurred after reperfusion. Fibrillation had to be terminated by a short period of perfusion with a high-K solution (17.0 mM). Panel c demonstrates the differences in electrode response that may occur during regional ischemia. One of the electrodes was located in the central zone, another in the normal region, and a third was located in the borderzone. At time zero flow through the LAD cannula was completely stopped and after 3 min 50 sec ventricular fibrillation started. The occlusion had to be discontinued and the heart was defibrillated by a high-K perfusion; this is shown by the increase of ∆EK recorded by the electrodes in the normal zone and the borderzone at t = 4 min. The time course of the increase of [K+]out of the central electrode was similar to the one during global ischemia, while the time course of the border electrode was intermediate between the central electrode and the one positioned in the normal zone.

Time course of extracellular potassium accumulation during ischemia. The time course of change of extracellular potassium activity during ischemia is shown in figure 2. Data from electrodes were pooled. In figure 2, a, the time course of ∆EK of five periods of global ischemia in five different preparations is
The seven hearts. The mean starting concentration was 4.8 ± 0.5 mM. The electrodes were classified in three groups: (1) normal zone electrodes showing less than 1 mV increase of ∆EK (corresponding to 0.2 mM K⁺) during ischemia (not shown), (2) borderzone electrodes, showing an increase of ∆EK of at least 1 mV and being located within 10 mm of a normal zone electrode, and (3) central-zone electrodes, showing an increase of ∆EK of at least 1 mV and being located at a distance of more than 10 mm from a normal-zone electrode. Since undetected normal myocardium may have been present close to the region where electrodes had been inserted, the possibility cannot be excluded that some of the electrodes, classified as central-zone electrodes, may in fact have belonged to the borderzone. Note that 95% confidence intervals are given in figure 2 instead of SD bars. Although there is some overlap between individual observations in figure 2, b, the 95% confidence intervals do not overlap from t = 4 min onward; from t = 2 min onward the means of the two groups are not included in each other's 95% confidence interval. This indicates that border and central electrodes belong to two distinct populations. Similarly, the 95% confidence intervals in figure 2, a, (global ischemia) do not overlap with those of figure 2, b (regional ischemia, central zone) from t = 4 min onward.

**Dispersion of extracellular potassium accumulation.**

Figure 3 shows an example of maps of distribution of ∆EK. Data from the electrodes are divided in classes of 10 mV ∆EK. In panels a (6 min test perfusion), and b (10 min global ischemia) nearly all measurements fall within one class. During test perfusion with a solution containing 8.1 mM K⁺, ∆EK

![Figure 1](image1.png)

**FIGURE 1.** Time course of ∆EK, recorded by three potassium-sensitive electrodes during a 6 min period of perfusion with a high-K⁺ (8.1 mM) containing solution (a), during a 10 min period of global ischemia followed by reperfusion resulting in ventricular fibrillation (bold arrow) and subsequent defibrillation by high-K⁺ perfusion with 17 mM K⁺ (thin arrow) (b), and during a short period of regional ischemia that after 3 min 50 sec ended in ventricular fibrillation, followed by reperfusion with high-K⁺ (thin arrow) (c). During regional ischemia one electrode (c) was situated in the normal zone, one (●) in the borderzone, and one (△) in the central zone.

shown. The mean starting value of [K⁺]_{out} was 4.7 ± 0.5 mM. The time course of ∆EK during regional ischemia is shown in figure 2, b. It incorporates data from 11 interventions (both LAD and CX occlusions) in seven hearts. The mean starting concentration was
was 18.1 ± 1.8 mV (n = 22), corresponding to 8.1 ± 0.6 mM K⁺; during global ischemia ΔEK was 24.9 ± 3.6 mV (n = 24), corresponding to 11.1 ± 1.6 mM K⁺. In contrast, in panel c (10 min regional ischemia of the LAD area), three different classes can be distinguished within the ischemic zone (central + border); in this case ΔEK within the ischemic zone was 16.4 ± 6.2 mV (n = 18), corresponding to a potassium concentration of 9.4 ± 2.0 mM. Electrodes a and b, only 7 mm apart, differed 19.8 mV (1.2 mV during test perfusion and 4.9 mV during global ischemia), corresponding to a 6.2 mM (0.7 mV during test perfusion and 1.8 mM during global ischemia) difference in [K⁺]ₗₒᵤᵗ, respectively.

Similar maps were drawn and maximal gradients were determined in seven hearts and 11 periods of regional ischemia (LAD and CX). Maximal gradients were defined as the maximal difference in ΔEK between two neighboring electrodes, normalized to 1 cm distance between electrodes. The mean maximal gradient was 28.7 ± 10.8 mV/cm (n = 11), which is equivalent to 8.33 ± 1.6 mM [K⁺]/cm. In nine of 11 periods of regional ischemia the maximal gradient was located in the borderzone; in the other two it was at a distance of more than 10 mm from a normal zone electrode.

Electrodes in the borderzone measured values of ΔEK intermediate between those measured in the central zone and those in the normal zone. For further analysis the dispersion of the change in [K⁺]ₗₒᵤᵗ (mM) for every single experiment was determined. The mean, SD, and variation coefficient (SD/mean) of the change in [K⁺]ₗₒᵤᵗ were calculated both for the central zone and for the borderzone at 7 to 10 min of ischemia. The variation coefficient (VC) in the borderzone was 0.58 ± 0.23 (mean ± SD, n = 11); the VC in the central zone was 0.35 ± 0.20 (mean ± SD, n = 11). This difference was statistically significant (p < .05). The mean VC during global ischemia was 0.31 ± 0.06 (n = 5); this was not significantly different from VC during regional ischemia in the central zone.

In four experiments global ischemia and test perfusion were performed in sequence. Table 1 lists the VCs for each separate intervention in those four hearts. Note that during test perfusion a certain amount of variation was still observed. This was interpreted as a methodologic error. In every single experiment VC was larger in global ischemia than in test perfusion: the mean VC of the two groups were not included in each other’s 95% confidence intervals.

The cause of dispersion of [K⁺]ₗₒᵤᵗ during regional ischemia. We considered the following possible causes of inhomogeneity of extracellular potassium accumulation during regional ischemia:

(1) The borderzone is equally ischemic (equal Po₂) to the central zone, but the differences in [K⁺]ₗₒᵤᵗ are brought about by washout. Causes of washout are collateral flow and “diffusion.”

(2) The borderzone is less ischemic (higher Po₂) than the central zone, giving rise to lesser degree of potassium loss in the borderzone.
TABLE 1

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>High K⁺ perfusion</th>
<th>Global ischemia, 5 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>m</td>
</tr>
<tr>
<td>1</td>
<td>22</td>
<td>3.7</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>4.5</td>
</tr>
<tr>
<td>3</td>
<td>19</td>
<td>11.5</td>
</tr>
<tr>
<td>4</td>
<td>17</td>
<td>3.4</td>
</tr>
</tbody>
</table>

Mean VC | 0.07
95% confidence interval | 0.06

n = number of electrodes; m = mean change in [K⁺]ₜₐₜ (mM); SD = standard deviation of change in [K⁺]ₜₐₜ (mM); Kc = potassium concentration control perfusion (mM).

We tested these possibilities by perfusing the normal tissue with oxygenated high-K⁺ perfusate from the moment of occlusion onward. This intervention alters the potassium gradient while maintaining normoxic perfusion of the normal zone.

Figure 4 shows data from one of three similar experiments. Panel b shows a potassium distribution map of ischemia in the LAD area after occlusion combined with high-K perfusion of the remainder of the heart. Inhomogeneity was less than after the control occlusion (figure 4, a). ΔEK in the borderzone was even higher than that in the central zone. This was completely opposite to what was seen with the control occlusion (figure 4, a). Moreover, some electrodes in the central zone measured larger ΔEK than those after the control occlusions. A second control occlusion after that in figure 4, b, was identical to that in figure 4, a (not shown).

These observations indicate that movement of extracellular K⁺ contributes significantly to the regional differences in potassium concentration. We tested whether collateral flow contributed to washout in two ways.

First, in eight experiments with subsequent occlusions of the LAD and the CX artery the borders coincided (not shown).

Second, in two experiments we occluded the LAD and the CX arteries simultaneously. Before the occlusions, the CX area was selectively perfused with a high [K⁺]-containing solution for 3 min. In this manner a K⁺ gradient was created, as illustrated in figure 4, b, but no pressure gradient existed as soon as both arteries were occluded. Figure 5 shows typical responses from an electrode situated in the borderzone of the LAD area (measured after a control LAD artery occlusion) and one located in the CX area. In the presence of a K⁺ gradient the electrode situated in the LAD borderzone showed a rapid increase in ΔEK after occlusion, as in the experiment in figure 4, b, while the electrode in the CX borderzone showed a decrease. Before the simultaneous occlusions this effect was not observed since the vascular beds were constantly perfused by the respective perfusion fluids.

Thus, in absence of collateral flow there is washout of potassium from the ischemic region, whether or not oxygenated tissue neighbors the border. This washout is related to the difference in [K⁺]ₜₐₜ in the ischemic and normal zones.

[K⁺]ₜₐₜ and monophasic electrograms. The absence of a local regenerative response in tissue surrounding an electrode is indicated by a monophasic QRST complex combined with the absence of a sharp negative deflection in the local electrogram (“block” electrogram). Depolarization of the cell membrane to

![FIGURE 4](http://circ.ahajournals.org/)

**FIGURE 4.** Maps of distribution of ΔEK after 5 min of occlusion of the LAD artery (a), and after 5 min of occlusion of the LAD artery combined with high-K⁺ (17.2 mM) perfusion of the remainder of the heart (b). In b the border between normal and ischemic tissue, determined in a control occlusion (a), is represented by a dotted line. Note that inhomogeneity in b is less than that in a, that the K⁺ gradient is reversed, and that electrodes both in the central zone and in the borderzone measure larger ΔEK than in the control occlusion (a). [K⁺] at t = 0 in panel a and b was 4.4 and 5.0 mM, respectively.
A critical level is related to the onset of inexcitability. Since the transsarcolemmal K⁺ gradient is in (near) equilibrium with resting membrane potential, ΔEK can be expected to be related to the onset of inexcitability as well.

In figure 6 direct current electrograms are plotted together with a ΔEK curve derived from an electrode in the borderzone: signals deteriorated progressively as ΔEK rose. After 8 min of occlusion a monophasic potential was encountered at a ΔEK of 7.2 mV ([K⁺]₉ of 6.6 mM).

The mean [K⁺]₉ at which monophasic potentials occurred was 8.6 ± 1.2 mM (11 periods of regional ischemia), which is equivalent to a local depolarization of 14 ± 5.4 mV. Inexcitability, expected to occur at a depolarization of approximately 25 to 30 mV, cannot be explained by this value.

Within a single experiment there was a large variation of ΔEK at which monophasic electrograms occurred; the mean ± SD was 4.3 ± 1.4 mV (n = 9). In two experiments only one electrode recorded a monophasic potential.

Both the large scatter within a single experiment and the low mean value of ΔEK at which monophasic
electrograms occur indicate that factors other than [K⁺]₉ influence the occurrence of monophasic electrograms. Activation maps suggest that the sequence of activation is of importance. This is illustrated in figure

FIGURE 5. Time course of ΔEK recorded by two electrodes, one in the borderzone of the tissue supplied by the LAD artery (○) and the other in the borderzone of the tissue supplied by the CX artery (△). Left, Changes during a 5 min LAD occlusion: a small rise of ΔEK is recorded by the LAD electrode. At t = 30 min (right) the CX artery is perfused with a high-K⁺ solution (16 mM), while the LAD artery is perfused with normal perfusion fluid. Three minutes later both the CX and the LAD artery are occluded, resulting in a small decrease in ΔEK recorded by the CX electrode (arrow) and a rapid increase of ΔEK recorded by the LAD electrode. This shows that the exchange of K⁺ between the borderzone and the normal zone during regional ischemia is not due to collateral flow.

FIGURE 6. Time course of ΔEK and ΔTQ during a 19 min period of regional ischemia recorded by a borderzone electrode; electrograms recorded by the same electrode are shown at the top. Note that after 8 min of occlusion a monophasic electrogram is recorded at a relatively low value of ΔEK, and that there is a subsequent decrease in ΔEK and ΔTQ depression coinciding with electrical recovery.
7, which shows the activation map corresponding to the ∆EK distribution map in figure 4, a. The area with a high [K+]_{out} (>9.6 mM K⁺ or >20 mV depolarization) prevents a neighboring area in the lower left side of the diagram from being reached by the activation front, although ∆EK is equally low at both sides of the “blocking” area, in the center of the map, where the tissue is effectively activated. In this particular map, the electrodes in the 10 to 20 mV class all recorded a ∆EK of 18 mV and less, those in the 20 to 30 class more than 23 mV. Thus, conduction block, manifest through monophasic electrograms, can occur in intrinsically excitable tissue that lacks an adequate stimulus.

[K⁺]_{out} and transient recovery. Figure 6 demonstrates the time course of potassium accumulation measured by an electrode located in the borderzone, together with electrograms at 0, 3, 5, 8, 9, 11, 15, and 19 min after the onset of ischemia. After 8 min the electrogram was monophasic but after 9 min it regained a small negative deflection until after 15 min a sharp negative deflection was present. Also, TQ depression and ST elevation decreased. After 19 min the signal became small, possibly as a result of electrical uncoupling, and 15 sec later ventricular fibrillation started (not shown). The electrical recovery closely followed the decrease of [K⁺]_{out}.

The decrease in [K⁺]_{out} began in the borderzone, but in later phases could also be observed in the central zone. In the experiments in which the occlusions lasted 10 min and less, 43 of 114 electrodes that were situated in the ischemic zones (both LAD and CX area) showed electrical recovery; of these, 37 (86%) also showed a decrease in [K⁺]_{out}. All of these electrodes were situated in the borderzone. The electrical recovery started at 7.7 ± 1.5 min of ischemia (n = 43). In four experiments with occlusions of 15 to 20 min duration, ∆EK measured in the central zone began to decrease as well. In these experiments a total of 61 electrodes were situated in the ischemic zones. In all four experiments all electrodes showed a decrease in ∆EK ranging from 1% to 100% of its maximal value reached. Sixteen electrodes (26%) recorded a small decrease in ∆EK of between 1% and 10%, and 16 recorded a decrease of 50% or more, including four in which normal [K⁺]_{out} was reached. Table 2 shows the relationship between maximal ∆EK and time of onset of decrease. The electrodes measuring smallest maximal ∆EK recorded an early decrease in ∆EK.

Of the 67 electrodes that measured [K⁺] during global ischemia (figure 2), five measured a decrease in ∆EK which was 17%, 15%, 5%, 4%, and 3% of the maximal values reached, respectively. None of the electrodes recorded electrical recovery.

TABLE 2

<table>
<thead>
<tr>
<th>Time of onset of decrease in ∆EK (min)</th>
<th>Maximal ∆EK (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–10 (n = 12)</td>
<td>8.8 ± 2.5</td>
</tr>
<tr>
<td>11–20 (n = 25)</td>
<td>9.4 ± 2.6</td>
</tr>
<tr>
<td>21–30 (n = 24)</td>
<td>9.4 ± 2.6</td>
</tr>
</tbody>
</table>

Differences tested with respect to 1 to 10 mV ∆EK.
|K^+|_{out} and TQ segment changes. Depression of the TQ segment of the local direct current electrogram during regional ischemia is caused by injury current flowing during diastole. This current is generated by a difference in resting membrane potential at either side of the border during regional ischemia. Since |K^+|_{out} is the major determinant of the resting membrane potential, an association is expected between TQ depression and ΔEK within the ischemic region during regional ischemia.

Figure 8 presents a typical example of a distribution map of TQ potentials obtained at the same moment during regional ischemia as the potassium distribution map of figure 4, a. Note that the electrophysiologic border and the potassium border coincide and that potassium peaks coincide with areas with maximal TQ depression.

Figure 9, a, shows a ΔEK-ΔETQ scattergram that combines data obtained from 114 electrodes at 4, 7, and 10 min durations of ischemia. No correlation existed between the two variables: 3 mV ΔETQ corresponded to a ΔEK ranging from 2 to 30 mV. When data were divided into a borderzone group and a central-zone group there was still considerable scatter: however, measurements derived from the central zone were mainly located in the lower right part of the scattergram.

The time course of local potassium accumulation and TQ depression of individual electrodes, however, showed a close association between the two variables over the entire period of ischemia (figure 6). Based on data from the electrode in figure 6 the correlation coefficient for ΔEK and ΔETQ was .93.

The median correlation coefficient for data from every electrode in all 10 min periods of regional ischemia (both of the LAD and the CX area) was .95 (n = 114); the tenth percentile was .75. This signifies a linear relationship between the two variables. This is illustrated in figure 9, b, for three different electrodes during the same period of regional ischemia. The trajectories were indeed straight lines, identical in upward and downward direction. However, the slope of the ΔEK-ΔETQ lines was different for different electrodes. The slope appeared to be dependent on the position of the electrodes: for electrodes situated in the borderzone it was 0.54 ± 0.40 (n = 62) and for electrodes situated in the central zone it was 0.30 ± 0.11 (n = 52). The difference between these two values is statistically significant.

Therefore, although there was a close correlation between ΔEK and TQ depression for every single electrode position, no overall correlation was found.

**Discussion**

Methodologic considerations. The determination of variations in extracellular potassium concentration during ischemia depends on the reliability of the methods used. The characteristics of the electrodes must be the same before, during, and after the experiment. The process of insertion of the electrodes might produce a small ischemic cylinder around the electrodes that acts as dead space, influencing the measurements. For the measurement to be accurate, the electrode space should...
be fully equilibrated with the surrounding interstitium. For these reasons we used thin electrodes, inserted them with atraumatic needles, and applied strict selection criteria to exclude those electrodes that had produced lesion (see Methods section). The response to a perfusion with a high-K solution shows that the electrode space equilibrates with the intravascular space in a relatively short time (figure 1).

A gradient of $K^+$ between intercellular clefts and the interstitium (and the electrode space) would cause our measurements to underestimate $[K^+]$ in the intercellular clefts; this would be particularly relevant in the presence of regional ischemia, when washout occurs even in the absence of collateral flow (figure 5). Consequently, this would affect the assumption that $\Delta EK$ equals local change of the resting membrane potential during regional ischemia and we would possibly underestimate local changes in resting membrane potential. However, we have shown that $K^+$ diffuses rapidly through the extracellular space over centimeters. It is not obvious why short distance diffusion between clefts and electrode space would be intrinsically slower, unless the clefts were effectively separated from the extracellular space during ischemia. Kline et al. measured $[K^+]_{\text{out}}$ in the intercellular clefts of a superfused canine Purkinje fiber. They showed a transient increase in $[K^+]_{\text{cleft}}$ during systole and a rapid return toward the concentration of the bath solution during diastole with the electrode inserted deeply into the tissue. These beat-to-beat fluctuations were not seen with the electrode in a more superficial position. We measured $[K^+]_{\text{out}}$ during diastole in a perfused preparation and although we cannot exclude the possibility of a gradient of $[K^+]_{\text{out}}$ between the intercellular clefts and the interstitium we would expect it to be small.

**FIGURE 9.** a, A $\Delta EK$-$\Delta ETQ$ scattergram of data from 114 electrodes at 4, 7, and 10 min of regional ischemia divided into a central zone group (●) and a borderzone group (○). b, Data from three electrodes in the same 19 min period of regional ischemia are plotted for every minute. When points coincided, a single point was given. Whereas there was no relationship between $\Delta ETQ$ and $\Delta EK$ when all data were considered, for any individual electrode there was a strict correlation, depending on its location (b). In b, dots represent data pairs at every minute of ischemia. The lines connecting the data points represent the time course of change in the two variables measured by one potassium electrode, with time $t = 0$ at the coordinate (0,0). All three electrodes measured transient recovery, which is shown by the "reversal" of the connecting lines toward zero.
Other sources of error include the interference of ions other than K⁺ ions, the calibration factor of the electrodes, and the correction of the K curves for baseline drift. Selectivity coefficients of the sensitive membrane have been reported to be 4000:1 for potassium over sodium ions, 18,000:1 over protons, 5000:1 over calcium ions, and 5000:1 over magnesium ions.³ Often a difference of 2 mV has been found between the Nernst constants determined before and after the experiment: 2 mV introduces an error of approximately 3%. The approximation of baseline drift in ΔEK with a straight line may add to inaccuracy, especially in longer lasting occlusions, when some deviation from a linear drift may occur.

Thus, electrode readings are affected by methodologic error. Injury caused by the insertion of the electrodes may have added to methodologic error in measurements during periods of rapid change in [K⁺]out, such as reperfusion¹⁹ or the initial rising phase of ischemia.²⁰ We therefore restricted analysis of dispersion of [K⁺]out to the steady-state phase of test perfusion and the plateau phase during ischemia.

Time course and distribution of potassium in ischemia. This study describes the time course of change in extracellular potassium during regional and global ischemia and the distribution of extracellular potassium.

Global ischemia. In global ischemia, the rise in [K⁺]out is faster and a plateau is reached at a higher concentration than in regional ischemia (figure 2). The time course of potassium accumulation during global ischemia in our experiments is in agreement with that described by others¹⁴, ²¹–²³ in different preparations. Methodologic error is partly responsible for the inhomogeneity in [K⁺]out observed in global ischemia (see above). Additionally, transmural inhomogeneity during ischemia has been demonstrated before.⁵, ²¹, ²⁴ Minor differences in the depth of the electrodes or the thickness of the ventricular wall might have contributed to the inhomogeneity in global ischemia. Since we did not thermostat the heart during global ischemia, a temperature gradient possibly amplified the effect of transmural inhomogeneity in [K⁺]out.

Regional ischemia. In association with regional ischemia, we found intermediate values of [K⁺]out (values between those in the central zone and in the normal zone) in the borderzone.

We have defined the borderzone on the basis of potassium measurements, excluding the normal zone, rather than on a visible, cyanotic border. Our choice of 1 cm as the width of the borderzone is arbitrary. This definition excludes the possibility that normal-zone tissue contributes to the larger variability in the borderzone. A large variability in [K⁺]out was found in a region extending 1 cm into the ischemic zone. Moreover, the influence of the normal zone was measured at a distance of at least 1 cm from the border (figure 4). The observations are not consistent with an abrupt border between the normal zone and the ischemic zone, as far as [K⁺]out is concerned, but indicate a gradual increase in [K⁺]out when moving into the ischemic region. Earlier measurement of metabolic, morphologic, and electrophysiologic variables in the borderzone indicate intermediate values in the borderzone.²⁵ These observations can be explained by a mixture of normal and ischemic tissue in the sample, the interdigitation of the vascular beds, and electrotonic coupling. However, in a critical review Hearn and Yellon²⁵ concluded that the transition between normal and ischemic tissue is probably accomplished over a distance of less than 2 mm and possibly in as little as the dimensions of one cell.²⁵ It is unlikely that the vascular beds interdigitate over distances of centimeters. This study demonstrates that potassium flows out of the ischemic region and that this flow establishes a true lateral border of [K⁺]out.

The marked inhomogeneity in [K⁺]out in the borderzone suggests inhomogeneity in electrophysiologic characteristics. Moderately depolarized cells have been shown to be in close proximity to severely depolarized cells.²⁶ Moreover, a decrease of refractory period in the borderzone and a simultaneous increase of refractory period in the central zone has been observed.⁷ Action potentials derived from the center of the ischemic zone have been observed to be of small amplitude and low upstroke velocity (“ischemic” action potentials), whereas in the borderzone the action potentials recorded have been of high amplitude and high upstroke velocity but of shorter duration (“hypoxic” action potentials).⁷ These findings suggest that in the regionally ischemic muscle areas exist that are anoxic but show a relatively small increase in [K⁺]out: indeed, the anoxic gradient has been shown to be much more steep than the potassium gradient.²⁷

In view of the large differences in ΔEK encountered in the regionally ischemic region one may assume that a relatively large area of increased excitability and increased conduction velocity (at [K⁺]out between 6 and 9 mM)¹⁹, ⁲⁸ is in close proximity to an area of slow conduction and decreased excitability (at [K⁺]out greater than 9 mM). This inhomogeneity may be of importance in the genesis of reentrant activity.

Mechanism of dispersion of potassium in regional ischemia. We have shown that the potassium gradient at the border during regional ischemia is determined by
the difference between the $[K^+]_{\text{out}}$ in the central zone and that in the normal zone. This gradient forces potassium to “diffuse” out of the ischemic region into the normal zone. Potassium may flow in the opposite direction (figure 4).

We did not establish whether this process is due to true diffusion or is aided by mixing of the extracellular space as a result of the contractions of the heart. Neither did our methods allow us to demonstrate an influence of the diffusion of oxygen into the ischemic zone on potassium accumulation. The distance over which oxygen can diffuse is on the order of tenths of millimeters, while in this study potassium appeared to “diffuse” over a distance of centimeters. Strong interdigitation of the normal zone and the ischemic zone may be present. In this respect it is noteworthy that there was no overlap between the borderzones after a CX occlusion and those after a subsequent LAD occlusion. Since electrode distance in our study was 4 to 10 mm we cannot assess the influence of oxygen on the $K^+$ accumulation at the lateral border. The possibility that ischemia is less severe in the borderzone therefore cannot be excluded.

A process of diffusion has been suggested by many authors, and can be inferred from the experiments of Harris and Cherry and Myers, who demonstrated a decrease of total potassium content in biopsy samples taken from the ischemic zone. In the latter study measurements were based on the wet weight of the samples, which could theoretically indicate an expansion of the extracellular space. The potential role of an osmotic gradient altering the interstitial space of the ischemic zone is not entirely clear.

The observation that during global ischemia the plateau is reached at a larger $\Delta EK$ than during regional ischemia, even within the central zone (figure 2), can be explained by the absence of washout toward a normal zone during global ischemia. The potassium movement that we observed during regional ischemia and the simultaneous increase in systemic $[K^+]$ has implications regarding pharmacologic interventions during ischemia, suggesting that drugs applied to the circulation can penetrate the ischemic muscle.

$[K^+]_{\text{out}}$ and transient electrical recovery. If the potassium movement from the extracellular compartment of the ischemic zone toward that of the normal zone is greater than the net loss of $K^+$ from the ischemic cells into the extracellular space, a decrease of $[K^+]_{\text{out}}$ can be anticipated. We showed that a normalization of $[K^+]_{\text{out}}$ begins in the borderzone and proceeds toward the central zone. The decrease in local $[K^+]_{\text{out}}$ was strongly correlated with transient recovery of electrical activity.

Other authors have reported transient recovery at times varying from 7 min to 1 hr after onset of ischemia. Our study demonstrates that the moment at which transient recovery is observed depends on the relative position of the recording electrode to the border and the magnitude of the ischemic zone. A decrease in extracellular $K^+$ will occur later in the center of a large ischemic zone than in a small ischemic area.

An alternative possible explanation for the transient decrease in $[K^+]_{\text{out}}$ after the initial increase in ischemia is reuptake of potassium into the cell. There have been reports in the globally ischemic rabbit interventricular septum that this might occur. The same mechanism could contribute to the lateral gradient of potassium during regional ischemia. Our experiments do not allow the quantitative assessment of cellular reuptake of $K^+$ during ischemia. During global ischemia, however, we did not observe a decrease in potassium after the initial increase such as was observed in regional ischemia; therefore, cellular uptake of potassium seems to be of lesser importance than the flow of potassium through the extracellular space in creating a lateral potassium gradient. If such a mechanism were operative it would be a special feature of regional ischemia in our experiments. Finally, an explanation for a transient decrease in $[K^+]_{\text{out}}$ after an initial increase during ischemia could be found in the decreased amount of time inexorable tissue spends in the depolarized state during systole, thereby reducing efflux of $K^+$ from the ischemic cells. Evidence in support of an association between the time course of extracellular potassium accumulation and the duration of the action potential has been reported.

The occurrence of monophasic electrograms. The occurrence of monophasic electrograms during regional ischemia could only be accounted for in part by the local $\Delta EK$, which is a measure of the change in the local resting membrane potential. Obviously other factors must play a role. These factors include acidosis, hypoxia, the lack of glucose, altered lipid metabolism, and free radicals. Our results show that areas that are only slightly depolarized still exhibit monophasic electrograms; these areas are intrinsically excitable but lack an adequate stimulus. A similar mechanism was demonstrated by Antzelevitch and Moe in superfused papillary muscle and by Janse and van Capelle in a computer model. The direction from which the ischemic area is invaded by the activation front possibly plays a role in the occurrence of
monophasic electrograms (figure 7). It could mean that intrinsically excitable tissue is "shielded" from being activated by surrounding inexcitable tissue. This mechanism is not likely to play a major role in ischemic tissue in close proximity to normal tissue.

The possibility exists that ischemic cells become less excitable for a given level of membrane potential when K+ conductance increases, for example by activation of ATP-sensitive K+ channels.43 However, the ATP-sensitive channels only become activated at ATP levels of 0.2 mM or less. In early ischemia ATP levels are considerably larger,22 and thus the mechanism mentioned above is likely to play a role only in later stages of ischemia, when cells are irreversibly injured.

\[ [K^+]_{out} \] and TQ depression

Severity of ischemia. In the pig heart collateral flow is known to be little or absent,13 and the borderzone is as oxygen deprived as the central zone,21 but for a small rim of several hundreds of microns. Still, very different \([K^+]_{out}\) values can be measured in the presence of regional ischemia. Therefore, \([K^+]_{out}\) is not a good estimator of the severity of regional ischemia. However, the electrophysiologic consequences of ischemia result mainly from changes in excitability, refractory period, and conduction; these changes are mediated by changes in the resting membrane potential.9 Large differences in local resting membrane potential have been reported,7 and in this sense \([K^+]_{out}\) can be thought of as an index of the amount of ischemic injury.

The relationship between local \(\Delta EK\) and the local change in resting membrane potential has been established in a globally ischemic preparation.14 We have shown that washout of potassium contributes to the differences in local potassium concentration observed in association with regional ischemia. This implies that intracellular potassium loss during regional ischemia is higher than that during global ischemia and that the amount of depolarization is possibly larger than that expected on the basis of \([K^+]_{out}\) alone, especially in longer lasting regional ischemia. However, this effect is not likely to play a major role in short-lasting occlusions.

K-TQ relationship. The amount of TQ depression in regional ischemia has been used as an indirect measure of the severity of ischemia.44 We have shown that TQ depression does not correlate with \(\Delta EK\) (figure 9, a), and Johnson et al.45 failed to find a correlation between \([K^+]_{out}\) and TQ depression. From a certain amount of TQ depression a corresponding amount of \(\Delta EK\) cannot be determined (figure 9). However, when single electrode positions were considered we found a linear relationship between local \(\Delta EK\) and local \(\Delta ETQ\); this relationship varied with the relative position of the recording electrode to the border. These findings corroborate the work of Holland and Brooks.17, 46 In their work, the distance between the recording electrode and the border and the size and the configuration of the ischemic area appeared to influence the amount of TQ depression. Additionally, tissue anisotropy47 or local differences in the changes in intracellular and extracellular resistances during regional ischemia could modulate the relationship between extracellular potassium concentration and TQ segment changes in regional ischemia.

We gratefully acknowledge the skillful assistance of Charles N. W. Belterman, Cees A. Schumacher, Ton Baartscheer, and Wim L. ter Smite.

References

Distribution of extracellular potassium and its relation to electrophysiologic changes during acute myocardial ischemia in the isolated perfused porcine heart.
R Coronel, J W Fiolet, F J Wilms-Schopman, A F Schaapherder, T A Johnson, L S Gettes and M J Janse

Circulation. 1988;77:1125-1138
doi: 10.1161/01.CIR.77.5.1125
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1988 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/77/5/1125

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/