The "Border Zone" in Myocardial Ischemia
An Electrophysiological, Metabolic, and Histochemical Correlation in the Pig Heart

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SUMMARY Regional ischemia was produced in isolated perfused pig hearts and in hearts in situ, by clamping the left descending coronary artery. Intramural and epicardial DC electrograms were recorded from multiple, regularly spaced sites in the central ischemic and border zones and in the normal myocardium. Subepicardial transmembrane potentials were recorded with floating microelectrodes. Transmural tissue biopsies were obtained with a drill from the sites of extracellular potential measurements at various times after coronary occlusion. Tissue levels of adenosine triphosphate (ATP), creatine phosphate (CP), and lactate were determined in this tissue. Glycogen distribution was assessed histochemically. Early ischemic changes are T-Q depression (1.5 minutes), S-T elevation (4 minutes), unresponsiveness (8 minutes), fall of CP (low and stable after 4 minutes), and rise in lactate (doubled within 10 minutes). Electrical activity temporarily returns between 30 and 50 minutes, while CP further decreases and lactate increases. After 2 hours, T-Q and S-T potentials have decreased, and cells have become unresponsive in the central ischemic zone; levels (in μmoles per gram dry weight) for CP and ATP are <1, for lactate circa 240. In the electrical border zone, intermediate metabolic values are found. No transmural electrical or metabolic gradients are present. In the border, zones with normal glycogen content interdigitate with zones depleted of glycogen. If occlusion is released after 2 hours, ATP, CP, and lactate levels do not change in central and border sites. In the border during reperfusion, cells with nearly normal transmembrane action potentials are in close proximity to unresponsive cells with low resting membrane potentials. These findings suggest that the ischemic border is composed of interdigitating normal and ischemic zones sharply demarcated from each other. Circ Res 44: 576-588, 1979

MEASUREMENT of S-T segment deviation is used to assess the extent and severity of ischemic injury to the myocardium. The problems associated with this technique have recently been discussed (Muller et al., 1978). Acute ischemia leads to a decrease of resting membrane potential and to a change of the action potential of the myocardial cell. The instantaneous differences in transmembrane potentials give rise to flow of extracellular current which determines the T-Q and S-T segment deflections in the intramural and epicardial extracellular electrograms. In the pig, acute coronary occlusion leads to electrical changes that show a regular transmural distribution. As a consequence of this distribution, the degree of local electrical change can be assessed from the extracellular electrogram. In addition to the consistent spatial distribution, the electrical changes also follow a characteristic time course (Kleber et al., 1978). In the pig, an electrophysiological border zone can be found in which the potentials change gradually from normal to ischemic. The ischemic extracellular complexes show maximal T-Q depression and S-T elevation, and, depending on the time after occlusion, transmembrane potentials are absent or have a very small amplitude (Kleber et al., 1978). The recent study by Hearse et al. (1977) showed that across such an electrophysiological border zone there is a sharp fall in high energy phosphate content, a decrease in flow, and an increase in lactate. Thus, an ischemic border zone can be found interposed between a central ischemic area and normal myocardium. In this border zone, intermediate values of various parameters for ischemic damage are measured. Such a border zone could in principle consist of cells that are homogeneously injured, but are injured less than the cells in the central area. Alternatively, the border zone could be composed partly of normal cells and partly of ischemic cells as severely damaged as the cells in the central area. The literature offers conflicting points of view. Marcus et al. (1975) found, on the basis of flow analysis, no evidence for a geometrically well-defined border zone of moderate ischemic myocardium surrounding and separating severely ischemic myocardium from normal tissue after a 5-minute occlusion in the
dog heart. In 24-hour infarctions, Hirzel et al. (1977) found in the dog heart a sharp demarcation between normal tissue and tissue in which creatine phosphokinase depletion was complete. Barlow et al. (1977) found a border zone that appeared as a patchwork of ischemic and normally perfused tissues sharing sharp interfaces. On the other hand, in the studies of Cox et al. (1968), a border zone in the dog heart was found surrounding severely ischemic tissue; damage in this border zone was confined to mitochondrial swelling. Also, Hearse et al. (1977) concluded from their findings that it was most likely that the border zone consisted of homogeneously damaged cells in which the damage was less severe than in the central ischemic zone.

The aim of this study was to obtain a better insight into the border zone by performing epicardial and intramural mapping of the changes in T-Q and S-T segments during the first 2 hours after coronary artery occlusion, and to relate these changes to other parameters of ischemic injury, such as tissue depletion of energy-rich compounds, lactate accumulation, and the changes in distribution of glycogen. A special attempt was made to compare the electrophysiological characteristics, metabolic state, and histochemical appearance of both the central ischemic area and the border zone during coronary occlusion and after reperfusion. Our hypothesis was that, in the case in which the central ischemic area was surrounded by a homogeneous shell of ischemic but potentially still viable cells, this shell should recover upon reperfusion following an occlusion that lasted long enough to produce irreversible damage in the central area. If the border zone consisted at that time partly of dead and partly of normal cells, upon reperfusion no improvement should appear.

**Methods**

Pigs weighing 18–25 kg were anesthetized by an intravenous injection of sodium pentobarbital (20 mg/kg), and the thorax was opened by a midsternal incision. Approximately 1 liter of venous blood was taken from the pig and mixed with a modified Tyrode's solution, yielding 2 liters of perfusion fluid. The heart was rapidly removed and fixed to the perfusion system and the composition of the perfusion setup where it was perfused according to the Langendorff method. The exact description of the perfusion system and the composition of the perfusion fluid are given elsewhere (Downar et al., 1977). The heart was driven above the spontaneous frequency of the sinus node by bipolar stimuli applied by means of a pair of electrodes fixed to the right atrium. Basic cycle lengths varied from heart to heart between 420 and 500 msec. Ischemia of the anterior wall and the septum was produced by clamping the anterior descending branch of the left coronary artery proximal to the origin of the first diagonal branch.

**Recording of Intramural DC Electrograms**

Polyethylene tubes (0.6 mm in diameter) served as intramural electrodes (see Fig. 1). The tube was about 10 cm long and was separated into two compartments by a small deposit of epoxy, which isolated the two compartments electrically from each other. A fine cotton thread was inserted into each compartment, which was filled with isotonic saline. The cotton wick was in contact with the extracardiac space via a small hole. The holes were made in such a way that when the tube was inserted into the ventricular wall, they were respectively 4 and 8 mm from the epicardial surface. A third cotton wick served as epicardial electrode. It was inserted into another polyethylene tube, which was glued to the tube containing the 4-mm intramural wick. The end of the cotton thread was wrapped around the intramural tube and was therefore in contact with the epicardium at the exact site overlying the intramural recording sites. The tube recording at 8 mm from the epicardial surface was passed through the left atrioventricular orifice to the outside of the heart. There, all tubes were inserted into saline-filled holes, which were drilled in a Perspex plate. To record the epicardial and intramural DC extracellular electrograms, a hand-held cotton wick inserted into a saline-filled polyethylene tube was used, which recorded the potentials in each saline-filled hole. The saline in this wick electrode was in contact with a chlorided silver wire, which was
connected to a high input impedance buffer amplifier. A differential input DC amplifier measured the potential at this wick with respect to a relative zero potential obtained from another wick electrode fixed to the aortic root. To ensure a precise geometrical arrangement, a thin rubber membrane was perforated to obtain a grid of holes of 0.7-mm diameter with a distance apart of 4 mm. This membrane was sutured onto the anterolateral part of the left ventricle, and the intramural electrodes were inserted through the holes, in single or parallel rows. The total number of electrodes inserted varied in the different experiments from 10 (30 recording sites) to 21 (63 recording sites). To assess the relationship between extracellular electrograms and transmembrane potential, floating microelectrodes were used in some experiments to record intracellular potentials from the subepicardium. The technique has been described earlier (Kleber et al., 1978; Downar et al., 1977). Three experiments were performed on the in situ heart. In these experiments, intramural recordings were made at one depth only (4 mm).

**Determination of Metabolic and Histochemical Changes**

To obtain tissue biopsies, we used a dental drill to which a sharp-edged cylindrical metal tube of 3-mm diameter had been attached. The intramural electrode was withdrawn from the heart, and the biopsy was taken, with the metal tube placed around the insertion opening of the electrode. Suction was applied during the drilling, which lasted about 1 second, and the biopsy was put into a container filled with liquid nitrogen by applying air pressure through the metal tube. Within 5 to 10 seconds after drilling started, the tissue was in the liquid nitrogen. In the in situ heart, the holes were filled with rubber stoppers trimmed to size to prevent loss of blood. In experiments in which the effects of reperfusion were studied, two rows of biopsies were taken: the lower row at the end of the occlusion and the upper row (parallel to the lower) at the end of the reperfusion period. The biopsies either were used for biochemical determinations, or were processed for the histological determination of glycogen distribution. In experiments in which the border zone was investigated before and after reperfusion, large biopsies 1 cm in diameter were obtained for histochemical studies.

For biochemical determination, the biopsies were freeze-dried overnight and weighed after careful removal of the adhering blood. The dry weight of the biopsies ranged from 15 to 20 mg. The dry tissue was deproteinized with 4% perchloric acid and centrifuged. The supernatant extract was neutralized with potassium phosphate-KOH to pH 7.0. Creatine phosphate (CP), adenosine triphosphate (ATP), and lactate were determined according to standard enzymatical procedure (Lamprecht et al., 1970) on a Beckman model 24 spectrophotometer. The results were expressed as μmoles per gram dry weight. For the histochemical determination of the distribution of glycogen, the tissue was cut in the cryostat into 7-μm sections. The sections were dehydrated and fixed in Carnoy solution, and then treated with periodic acid-Schiff (Pearse, 1968).

**Results**

**Changes in Extracellular Potentials and Tissue CP, ATP, and Lactate in the First 2 Hours after Coronary Occlusion**

Figure 2 shows the changes in epicardial and intramural DC electrograms in the first 2 hours after coronary artery ligation. Measuring sites were situated along a row, that was oriented from the ischemic toward the normal myocardium. Electrodes A to F were placed 4 mm apart, and at each site recordings were made at the epicardium and in the wall at depths of 4 and 8 mm. Electrode A was in the anterior wall next to the left anterior descending artery; electrode F was in the lateral wall of the left ventricle.

In the control situation, about 15 minutes after the last electrode has been inserted, all injury current caused by the insertion has disappeared, and T-Q and S-T segments are isoelectric (the straight line indicates the potential level at the aortic root). After 8 minutes of ischemia, both the epicardial and intramural recordings at A and B show fully developed T-Q segment depression (on the order of −10 to −15 mV) and S-T segment elevation (on the order of +15 to +25 mV). Intrinsic deflections have either disappeared or have become very small, indicating that local membrane responses are absent, or are small and probably not capable of propagating (Kleber et al., 1978). Electrode C is in the border zone: the magnitude of T-Q depression and S-T elevation is less than in A and B, and a large intrinsic deflection indicates that the local transmembrane action potentials have a large amplitude. Electrodes D, E, and F are in normal myocardium and show slight reciprocal T-Q segment elevation and S-T segment depression.

After 30 minutes of occlusion (Fig. 2B), intrinsic electrical activity has returned at all recording sites of electrodes A and B, and T-Q and S-T segment potentials have diminished in amplitude. After 2 hours, all potentials in A and B have become monophasic, indicating absence of local activity; the magnitude of T-Q and S-T potentials has substantially decreased, compared with the potentials recorded after 8 minutes. Throughout these 2 hours, the location of the electrical border zone has remained constant; the recordings of electrode C show in every stage ischemic changes (i.e., T-Q depression and S-T elevation), but to a lesser degree than in neighboring electrode B, and in all stages the presence of a sizeable intrinsic deflection indicates that the transmembrane action potential has a fairly large amplitude. The T-Q and S-T segments of electrodes D, E, and F were either isoelectric or showed slight reciprocal changes (T-Q elevation...
and S-T depression). After 2 hours of coronary artery occlusion, biopsies were taken from sites A through F. At A and B, lactate levels were high and CP and ATP levels were low; at C and D intermediate values were found, and levels at E and F were normal.

Figure 3 shows the results of three different experiments in which recordings were made from electrodes regularly inserted at 4-mm intervals, and in which biopsies were taken 10, 50, and 120 minutes after occlusion of the left anterior descending artery. It is evident that, with time, both T-Q and S-T segment potentials decrease, whereas the metabolic situation progressively worsens. Lactate levels within the ischemic area increase, but CP and ATP levels decrease with time. As is evident from Figures 2 and 3, and as was reported earlier (Kleber et al., 1978), no important transmural potential gradients exist at any one location in the different stages of ischemia.

In one experiment, 11 tissue biopsies were taken from the central area after a 2-hour occlusion. A drill of 4-mm diameter was used, and biopsies were divided into subepicardial and subendocardial halves. No differences were found in tissue content of CP, ATP, and lactate in the two halves. Subepicardial values were: CP, 1.3 ± 0.8; ATP, 0.8 ± 0.2; lactate 240 ± 6 μmol/g dry weight. Subendocardial values were: CP, 1.1 ± 0.3; ATP, 0.5 ± 0.2; lactate, 232 ± 5 μmol/g dry weight.

**Time Course of Electrical and Metabolic Changes in the Early Stage of Ischemia**

Four experiments were undertaken in which recordings were made in the ischemic area only. The electrodes were not inserted in a regular row, but as closely as possible in a cluster, in the area between the anterior descending artery and one of the diagonal branches. Following occlusion of the artery, the electrodes were withdrawn one by one, after recordings had been made, at intervals varying from 30 to 60 seconds, and a biopsy was immediately taken from that site.

Figure 4 shows the results of one such experiment. In the ischemic area, the electrical signals after 1 minute show depression of the T-Q segment, indicating a decrease in resting membrane potential throughout the ventricular wall. The increase in R

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**FIGURE 2** A and B: Epicardial and intramural DC electrograms (at 4 and 8 mm from the epicardial surface) before and 8 minutes, 30 minutes, and 2 hours after occlusion of the left anterior descending artery. Electrodes A to F were in one row, separated by 4 mm. Electrode A was in the anterior wall of the left ventricle next to the left anterior descending artery, and electrode F was in the lateral wall. The straight line in the signals represents the potential of the aortic root. At the end of the 2-hour occlusion, transmural tissue biopsies were obtained with a 3-mm diameter drill at the electrode sites, and tissue levels of lactate, CP, and ATP are indicated in μmoles per gram dry weight.
Figure 3 Three experiments in which epicardial and intramural T-Q and S-T segment potentials were measured and transmural biopsies taken at the electrode sites after occlusions lasting 10, 50, and 120 minutes. Electrodes were placed in a row (see inset) and were separated by 4 mm. T-Q potentials are represented by unfilled circles, S-T potentials by filled circles. The moments of the cardiac cycle at which measurements were made are indicated in the upper left inset. Note the absence of significant transmural potential gradients at each site. In the lower half, tissue content of lactate, CP, and ATP at the electrode sites are indicated in μmoles per gram dry weight. Upward-pointing arrows indicate electrical border sites; downward-pointing arrows indicate metabolic border sites (see text). Note that in the first panel, metabolic measurements at the electrical border site were normal.

Figure 4 Epicardial and intramural DC electrograms from the central ischemic zone before coronary artery occlusion and at different intervals following occlusion, as indicated on the abscissa of the lower graph. After a recording had been made, the electrode was withdrawn from the tissue, and a transmural biopsy was immediately taken from that site. Tissue levels at these sites of CP, ATP, and lactate are given in μmoles per gram dry weight. Note the early development of T-Q depression and the fall in CP. Note also the changes in and absence of marked transmural T-Q and S-T gradients.

wave, apparent after 3 minutes, is due to a delay in local activation time. After 4 minutes S-T segment elevation appears, caused by a decrease in action potential amplitude and duration, and after 8.5 minutes the complexes have become monophasic, indicating absence of local electrical activity. After 25 minutes, small intrinsic deflections appear, indicating a temporary recovery in local electrical activity which starts to disappear again after 39 minutes. At this time, the magnitude of T-Q and S-T segment potentials has decreased (Kléber et al., 1978). The time course of the electrical changes was remarkably constant, as was the change in tissue levels of CP and lactate, as shown in Figure 5, in which the results of all four experiments are pooled. There is an exponential decrease in CP level, and low and stable levels are reached within 4-5 minutes, with a half-time of about 1 minute. Lactate content rises linearly at a rate of about 5 μmol/g dry weight per min. In contrast, ATP levels, although generally declining, are far more scattered than values for CP and lactate.

The Border Zone

An extracellular electrogram was considered to belong to the border zone: (1) when it showed T-Q depression and S-T elevation less than the recordings from the neighboring electrode located 4 mm nearer the ischemic center, and/or (2) when a large
intrinsic deflection was recorded, indicating the presence of large-amplitude action potentials while adjacent recordings moving toward the center showed absence of, or very small, intrinsic deflections (see Fig. 2). In Figure 3, the arrows pointing upward indicate electrophysiological border sites. When biochemical determinations showed values intermediate between ischemic and normal values, these sites were considered to be biochemical border sites and are indicated by downward-pointing arrows.

Figure 3 shows the variability in border zones in the different experiments. In the 10-minute occlusion experiment, no biochemical border was found, and the biopsy that electrophysiologically was border zone was biochemically normal. In the 50-minute occlusion experiment, only one site was found to be border zone both electrically and biochemically, and in the 2-hour experiment, the border included two successive sites. It is emphasized that in each individual experiment the electrophysiological border remained constant throughout the period of coronary artery occlusion (Fig. 2).

Table 1 shows the correlation between electrophysiologically and biochemically determined border sites in all experiments (both in isolated hearts and in hearts in situ). There is no complete overlap, since the electrophysiological definition seems to overestimate the ischemic damage. In four cases, a site was identified electrophysiologically as ischemic and biochemically as border; in another four cases, biochemically normal values were found at sites that were electrophysiological border sites. In no case was a site electrophysiologically normal and biochemically ischemic, or biochemically normal and electrophysiologically ischemic.

Histochemical Determination of Glycogen Distribution

Within the central ischemic area, glycogen gradually disappears with time. After an occlusion of 45 minutes, there is a patchy distribution of glycogen; areas with total cellular glycogen depletion intermingle with areas in which the cells show varying amounts of glycogen. When coronary artery occlusion has lasted for longer than 1 hour, glycogen depletion in the central ischemic area is complete and homogeneous (see Fig. 6A). Only some of the cells, located around blood vessels and Purkinje fibers, still show some glycogen granules. In the earlier phases, at up to 45 minutes of occlusion, the electrophysiological border zone and the central ischemic area show a similar distribution of glycogen. In other words, the border zone could not be distinguished from the central area by the criterion of glycogen distribution. After 1 hour of occlusion, when the central ischemic area showed a complete depletion of glycogen, the electrophysiological border zone still exhibited the patchy distribution of glycogen. However, it now appeared that fully stained cells were usually juxtaposed to completely empty cells, but small areas could be found where cells with an intermediate glycogen content lay between darkly-stained and empty cells (Fig. 6B). The demarcation between glycogen-depleted and glycogen-containing cells does not run in a straight line from endocardium to epicardium. As shown in Figure 7, the distance parallel to the surface where both zones are interdigitated is of the order of 4 mm.

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<tr>
<th>Biochemical</th>
<th>Electrical</th>
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<td>Ischemic</td>
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<tr>
<td>Ischemic</td>
<td>85</td>
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<td>Border</td>
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Reperfusion

When coronary artery occlusion lasted 15 minutes, reperfusion resulted in a rapid normalization of T-Q and S-T segment potentials and a return to baseline levels for CP, ATP, and lactate within 15 minutes. When the occlusion lasted 1 hour, reperfusion for a period of 1 hour led to a partial recovery of electrical signals and biochemical parameters in the central ischemic zone. In areas where during occlusion the extracellular complexes were monophasic, reperfusion resulted in reappearance of intrinsic deflections, indicating that after 1 hour of occlusion not all cells were irreversibly damaged. CP content changed from an average value of 0.1 μmol/g dry weight at the end of 1 hour of occlusion to an average of 13.6 μmol/g dry weight after reper-
fusion. Lactate decreased from an average of 235 to 165 μmol, whereas ATP did not change (2.9 μmol during occlusion, 2.8 after reperfusion). In the border zone, lactate and ATP remained unchanged and CP increased. Sections stained for glycogen showed that in the central area, completely depleted during the occlusion, reperfusion resulted in some return of glycogen, although the greater part of the tissue remained glycogen-depleted. When coronary occlusion was maintained for 2 hours, reperfusion did not result in recovery in the central ischemic area or in the border zone. In Figure 8, extracellular and transmembrane potentials are shown. The recordings during occlusion were made between 1 and 1.5 hours after coronary occlusion, and those during reperfusion were made 30-40 minutes after release of the occlusion. Potential profiles during T-Q and S-T segments are depicted for a lower, parallel row after 2 hours of occlusion and for sites A through F after 1 hour of reperfusion. Values for CP, ATP, and lactate are plotted as obtained from the biopsies taken before reperfusion and after a 1-hour period of reperfusion showed essentially the same values for CP, ATP, and lactate.

In Figure 9, transmembrane potential and extracellular complexes are shown as recorded in another experiment during reperfusion following a 2-hour occlusion. Here an attempt was made to impale from different cells at the same site. At sites to the left of site A, extracellular complexes were monophasic both after 2 hours of occlusion and during the 1-hour period of reperfusion, and no transmem-
brane action potentials could be recorded. Multiple impalements at site A revealed the presence of partially depolarized cells exhibiting no action potentials in close proximity to cells showing abnormally short and small action potentials. This is in keeping with the multiple, fragmented intrinsic deflections in the extracellular complex recorded at site A. At site B, different action potentials were also found with multiple impalements, but amplitudes were larger and duration longer than at site A. At site C, normal action potentials were recorded.

Figure 10 shows the pooled results of the 16 experiments in which successive microelectrode impalements were made at ischemic, border, and normal sites, separated by 4 mm. The recordings were made at times varying from 15 minutes to 2 hours after the beginning of occlusion and after a 1-hour period of reperfusion following a 2-hour occlusion. It is evident that, in the border zone, values for resting membrane potential, action potential amplitude, and action potential duration are widely scattered. Values identical to those in the normal area as well as values characteristic for ischemic potentials were found.

When the biochemical determinations made in isolated hearts are summarized (Fig. 11), it can be seen that, with longer-lasting coronary occlusion, CP and ATP levels decline and lactate accumulates, both in the central ischemic area and in the border zone. After a 1-hour period of reperfusion following a 2-hour occlusion, the metabolic state of both the central area and the border zone is essentially the same as at the end of the occlusion, and the same difference exists between central ischemic values and border zone values.

**In Situ Experiments**

Three experiments were carried out on hearts in situ in which recordings were made at epicardial and intramural sites at 4-mm depth at successive sites separated by 4 mm, and biopsies were taken after occlusions of the left anterior descending ar-
FIGURE 10 Values of resting membrane potential, action potential amplitude, and action potential duration at ischemic (I), border (B), and normal (N) sites. Results of 18 experiments are pooled. Filled circles indicate measurements made between 15 minutes and 2 hours of occlusion; unfilled circles are values found after a 1-hour period of reperfusion following a 2-hour occlusion. In ischemic and border areas some cells showed no response (amplitude 0) at low resting membrane potentials. These measurements are not represented in the graph depicting action potential duration. Note that in the border zone, both during occlusion and after reperfusion, all gradations between normal and unresponsiveness occur.

Discussion

Our findings show that in the first 3 minutes after coronary artery occlusion tissue CP falls rapidly and resting membrane potential decreases, as is evident from the depression in the T-Q segment (see Figs. 2 and 3). Only after 4 minutes does S-T elevation, caused by a decrease in action potential amplitude, become apparent, and significant changes in ATP and lactate occur. The time course of change in CP, ATP, and lactate following coronary artery occlusion in our experiments closely resembles the findings by Braasch et al. (1968) in the dog. The rapid decline in CP content probably indicates a drastic fall in ATP-generating and ATP-consuming processes. The fall in CP could thus be interpreted as a measure of the energy depletion of the cell. Remaining to be established is whether there is a causal relationship between the fall in CP [and thus in phosphate potential (Slater, 1969)] and the decrease in resting membrane potential. It is known that local extracellular K+ rises between 5 and 15 minutes after coronary artery occlusion to values around 12 mM (Hill and Gettes, 1977), which would explain the decrease in resting membrane potential. The increase in extracellular K+ concentration is due to a net loss of intracellular K+, which accumulates in the extracellular space because it cannot be washed out by the coronary circulation. The net intracellular K+ loss can be due to an...
inhibition of active inward K⁺ transport and to an increase in passive outward movement. Calculations by Johnson (1976) indicated that a complete arrest of active ion transport would raise the extracellular K⁺ concentration by about 0.5 mm/sec. Whether the observed fall in CP is sufficient to inhibit active K⁺ transport to such an extent as to explain the rise in extracellular K⁺ cannot be determined from our experiments. A rapid fall in intracellular pH might primarily be required to inhibit active transport (Williamson et al., 1976).

This study demonstrates that the absolute magnitude of T-Q and S-T segment potentials at a particular site does not indicate the severity of the metabolic changes of the underlying tissue. S-T and T-Q potentials reach their maximal values at 7-15 minutes after coronary artery occlusion and decrease with time thereafter, whereas the metabolic situation progressively deteriorates. The reason for the decline in T-Q and S-T segment potentials may be 2-fold: (1) the transient recovery of electrical activity of ischemic cells between 20 and 60 minutes, and (2) additional electrical uncoupling of the ischemic cells so that, despite intracellular potential gradients between ischemic and nonischemic cells, current flow responsible for T-Q and S-T potentials is impeded (Kleber et al., 1978). The transient recovery in electrical activity has been noted before (Downar et al., 1977; Scherlag et al., 1974), and could have been due to an increased collateral flow. In that case, one would have expected a simultaneous improvement in the metabolic situation. Our results show that, at the time of electrical improvement, ATP and CP content has decreased and lactate content has increased, compared to the period after about 10 minutes of coronary artery occlusion when electrical activity was absent in the central ischemic area. A possible explanation for the transient recovery of electrical activity is that the concentration of substances that leak out from the ischemic cells and accumulate in the extracellular space gradually diminishes with time. For example, it is possible that K⁺ loss from ischemic cells diminishes during the period of unresponsiveness. Also, a gradual diffusion of K⁺ (and other substances) toward the normal myocardium may alter the composition of the extracellular fluid in the ischemic area to such an extent that, despite a large energy depletion, the resting potential of ischemic cells is sufficiently restored so that they are still capable of generating (abnormal) action potentials. It is known that a simple flush with saline into the occluded artery can give rise to the temporary reappearance of action potentials in previously unresponsive cells (Downar et al., 1977), and can lead to a decrease in S-T elevation in the extracellular electrogram (Bodenheimer et al., 1976). Differences in venous drainage pattern of the ischemic area may thus give rise to differences in T-Q and S-T segment potentials in different hearts, although the metabolic condition may be the same.

Other studies had already indicated that there is no direct relationship between magnitude of S-T elevation and other parameters indicating ischemic injury. Karlsson et al. (1973) found no direct relationship between magnitude of epicardial S-T elevation and degree of lactate accumulation and severity of ATP depletion. Smith et al. (1975) found areas of myocardium with a flow of less than 10 ml/min per 100 g which showed no epicardial S-T elevation. However, these experiments were performed on dog hearts, in which ischemic changes may not have been transmural (Holland and Brooks, 1975, 1977). Our results indicate that no transmural potential gradient exists in the extracellular space at one location (Kléber et al., 1978), and that no metabolic gradient could be detected 2 hours after coronary occlusion. In the pig, therefore, the infarct is truly transmural.

The Border Zone

Many different techniques have been used to determine a border zone where the changes brought about by coronary artery occlusion were present, but to a lesser degree than in the central ischemic area. These changes are reduced local blood flow at levels intermediate between those of the normal myocardium and the central ischemic zone (Smith et al., 1975; Beker et al., 1973), morphological changes pointing to mitochondrial swelling with intact cellular architecture (Cox et al., 1968), altered tissue ratios for K⁺/Na⁺ and for Mg²⁺/Ca²⁺ (Lie et al., 1975), decreased myocardial oxygen tensions stabilizing at relatively high levels (Sayen et al., 1961), and T-Q and S-T segment changes (Kléber et al., 1978). It has not been determined whether all these changes are present at the same sites, and it is conceivable that the dimensions of the border zone differ according to the parameters used. In those studies in which dimensions are given, there is good agreement. Thus, in the pig heart, the zone in which blood flow changed from very low to normal values had an average length of 7.5 mm (Brooks et al., 1975), and the zone where extracellular potentials changed from monophasic complexes with maximal T-Q depression and S-T elevation was of the order of 9 mm (Kléber et al., 1978).

The study most closely resembling ours is that by Hearse et al. (1977), in which the border zone in dog hearts was determined by measurement of blood flow, metabolic, and electrocardiographic changes for coronary artery occlusions about 20 minutes in duration. Over an area of 8-15 mm, gradients were found in local blood flow, lactate content, high energy phosphate content, and S-T segment potentials. All these correlated quite well with each other. The authors recognized that the finding of an intermediate value for flow and metabolic state could be explained when the border tissue was either a mixture of normal and ischemic cells or was uniformly composed of cells with an
intermediate degree of change. Although the technique used did not allow differentiation between these possibilities, the authors supported the concept of a border zone consisting of cells with an intermediate degree of injury, since interventions such as glucose-insulin-potassium administration were particularly effective (i.e., decreased S-T segment potentials and improved K⁺/Na⁺ ratios and high energy phosphate content) in the border zone (Opie and Owen, 1976).

Our findings support the concept of a border zone consisting of a mixture of normal and ischemic cells. The arguments for this statement are as follows. When coronary artery occlusion lasts longer than 1 hour, a marked contrast can be seen in histochemical sections between cells affected by ischemia and cells unaffected (Yokoyama et al., 1955). We found that, in the border zone, areas of glycogen-depleted cells interdigitate with areas filled with glycogen, indicating that the cell population in a tissue cylinder of 3-mm diameter (the diameter of the biopsy drill) is not homogeneous, so that biochemical determinations from a border biopsy are measurements from more than one type of cell. Furthermore, the demarcation between cells filled with glycogen and empty cells was usually sharp, although sometimes small groups of cells were found with an intermediate glycogen content. Thus, after 1 hour of occlusion, there appeared no spectrum of changes in glycogen content; cells were either full or empty. We recognize that absence of glycogen does not indicate whether a cell is reversibly damaged, and it is possible that a glycogen-depleted cell in the central ischemic area is more severely damaged than a glycogen-depleted cell in the border zone. However, our reperfusion studies speak against such a possibility. Reperfusion after 2 hours of occlusion resulted in a metabolic gradient that was practically identical to that occurring during occlusion. This does not mean that at a cellular level no changes occurred on reperfusion. Some cells may have recovered and others may have died, but the overall characteristics of the border zone did not change. Microelectrode recordings showed that in the subepicardium of the border zone, both after a sufficiently long occlusion and after reperfusion, unresponsive cells were in close proximity to cells exhibiting a nearly normal transmembrane action potential. No specific border zone action potential was found; as shown in Figure 10, all gradations between normal resting potential and action potential and unresponsiveness at low resting membrane potential levels occurred. The fact that action potentials in the border zone are not normal does not necessarily indicate that the cell recorded from is injured. Electrotonic influences from nearby ischemic cells may shorten the action potential and decrease resting membrane potential and action potential amplitude. However, we cannot completely exclude the possibility that the border zone contains some cells that have an intermediate degree of injury. Cells with highly abnormal action potentials, such as some cells at site A in Figure 9, might even after reperfusion be partially injured.

No reperfusion studies in the in situ heart were undertaken. Although during ischemia the initial time course of electrical and biochemical changes was similar in both the isolated and in situ heart, the final unresponsive stage was reached earlier in the heart in situ. One may speculate that after a 2-hour occlusion, the ischemic cells in the in situ heart are at least as severely injured as those in the isolated heart.

Thus, after a 2-hour occlusion, most of the cells in the border zone affected by ischemia are dead. The position of the electrophysiological border zone in an individual experiment is constant. This does not necessarily indicate that the fate of a cell in the border zone is determined in the first few minutes after coronary artery occlusion. Interventions in the early stages might have altered the ultimate destination of border zone cells and changed the point in time at which those cells affected by ischemia are irreversibly damaged. Our studies do suggest that interventions designed to restore cell function should be performed in a very early phase of ischemia. It is conceivable that the presence of collaterals will influence the characteristics of the border zone. In the pig heart, preexisting collaterals are much less developed than in dog or human hearts (Schaper, 1971). Thus, the choice of experimental animal for studies designed to test the efficacy of hemodynamic or pharmacological interventions to decrease the size of the ischemic zone may be of crucial importance.

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References

Barlow, CH, Harken AH, Chance B: Evaluation of ischemic areas in cardiac tissues by fluorescence spectroscopy (abstr). J Mol Cell Cardiol 8(suppl): 14, 1977
Downar E, Janse MJ, Durrer D: The effect of acute coronary artery occlusion on subepicardial transmembrane potentials
in the intact porcine heart. Circulation 56: 217-224, 1977
Hearse DJ, Opie LH, Katzeff IE, Lubbe WF, van der Werff TJ, Feisach M, Boule G: Characterization of the "border zone" in acute regional ischemia in the dog. Am J Cardiol 40: 716-726, 1977
Schaper W: The collateral circulation of the heart. Amsterdam, North-Holland, 1971, pp 5-18
Williamson JR, Schaffer SW, Ford C, Safer B: Contribution of tissue acidosis to ischemic injury in the perfused rat heart. Circulation 53 (suppl 1): 3-14, 1976
The "border zone" in myocardial ischemia. An electrophysiological, metabolic, and histochemical correlation in the pig heart.
M J Janse, J Cinca, H Moréna, J W Fiolet, A G Kléber, G P de Vries, A E Becker and D Durrer

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