Absence of a Lateral Border Zone of Intermediate Creatine Phosphokinase Depletion Surrounding a Central Infarct 24 Hours after Acute Coronary Occlusion in the Dog

HEINZ O. HIRZEL, EDMUND H. SONNENBLICK, AND EDWARD S. KIRK

SUMMARY Myocardial creatine phosphokinase (CPK) activity was measured as an indicator of cell viability 24 hours after ligation of the left anterior descending coronary artery (LAD) in normal myocardium, the entire region supplied by the LAD, and individual samples from the border and center of the infarct. Tissue supplied by the LAD and delineated by dye was carefully dissected from normal tissue along the stained border. CPK activity in the ischemic myocardium was calculated by assuming normal CPK activity in normal myocardium interdigitating with ischemic tissue at the border. Normal tissue was marked prior to occlusion with microspheres injected into the left atrium, whereas the distal portion of the LAD was perfused separately with unlabelled blood from a reservoir. With this correction, the CPK activity in the ischemic tissue from the lateral border of the infarct was essentially the same as in samples from the center, whereas in the normal tissue immediately adjacent to the stained border was equal to values in remote normal myocardium. Thus, CPK depletion throughout the entire ischemic myocardium was nearly equal to CPK depletion in the center of the infarct. The uncorrected intermediate CPK levels in the individual samples from the border of the stained region correlated with the amount of normal tissue contaminating these samples. However, differences in CPK depletion across the heart wall resulted in the most depletion in the subendocardium and the least in the epicardium. Furthermore, coronary collateral blood flow measured 10 minutes after occlusion correlated well with the subsequent extent of CPK depletion.

TRADITIONALLY, infarcted myocardium is viewed as a region of central necrosis surrounded in a shell-like fashion by a substantial amount of ischemic but still viable tissue. Although there is an increasing awareness that the endocardial layers are more involved in the process than the epicardial ones, lateral border zones of intermediate tissue damage are implicit in this current description. This picture corresponds to the similar conception of the acutely ischemic region, where the flow has been found to decrease gradually from the surrounding normal myocardium through zones of intermediate flow levels to the deeply ischemic center of the region. This so-called border zone concept has been supported by observations of differences in the mechanical and the electrophysiological behavior of the center and the border regions of ischemic myocardium. Moreover, measurements of intramyocardial pressures of oxygen and the depletion of enzymes such as the CPK in the tissue would also agree with this view. Finally, pathological and histochemical studies have also provided evidence for the existence of border zones.

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This concept, however, has not remained unquestioned. In 1966 Linder published data suggesting a very steep reduction in blood flow taking place within a narrow border zone between normal and ischemic myocardium. More recently, Fischl et al. demonstrated that the blood flow in the acutely ischemic tissue was the same at the lateral borders of the ischemic region as in the center. Marcus and co-workers came to a similar conclusion on the basis of detailed analysis of the distribution of myocardial blood flow. Thus, the concept of a geometrically clearly defined "buffer zone" of moderately ischemic myocardium surrounding and separating severely ischemic from normally perfused myocardium has been seriously questioned.

The existence or emergence of border zones of intermediate flow following acute coronary occlusion and their location are of significance because they could represent the tissue which might be salvaged by proper treatment. Further, were such border zones to exist, they might lead to a central region of infarction surrounded by regions of reduced flow and patchy necrosis. Therefore, the present study was undertaken to explore the existence of zones of intermediate tissue damage surrounding a massive central infarct and to describe the topography of infarction 24 hours after ligation of the left anterior descending coronary artery (LAD) in the dog.

Methods

EXPERIMENTAL PREPARATION AND PROCEDURE

The study was conducted on a total of 53 mongrel dogs of either sex, weighing 18.0 ± 0.6 kg (mean ± 1 se). The
dogs were anesthetized with sodium pentobarbital (27.5 mg/kg, iv) and ventilated through a cuffed endotracheal tube with 100% oxygen and a positive pressure respirator. Under clean conditions a left-sided thoracotomy was performed, the heart exposed, and the LAD isolated below its first diagonal branch. A polyvinyl catheter was inserted into the left atrium through the appendage to permit the injection of radioactive microspheres and the measurement of left atrial pressure. Similar catheters were introduced via the right femoral artery into the thoracic aorta to measure systemic blood pressure and via the right femoral vein into the inferior vena cava for administration of drugs and fluid. The pressure, measured by means of Statham P23Db pressure transducers (Statham Instruments), were recorded continuously on a Beckman type SII multichannel oscillograph (Beckman Instruments). Heparin (5000 units, iv) was then administered, the LAD ligated, and a cannula inserted distal to the site of occlusion. Perfusion was subsequently continued with arterial blood from the left carotid artery through a large bore cannula and a polyvinyl tubing system (Fig. 1). The procedure of inserting the cannula into the LAD ordinarily caused an interruption in perfusion of no longer than 2-3 minutes. After flow was restored in the distal LAD, the tissue supplied by this portion of the vessel was perfused through the bypass system for a recovery period of approximately 20 minutes.

Two side arms in the bypass system led to a 50-ml reservoir containing a balloon. The system was arranged so that filling of the balloon resulted in a simultaneous emptying of the reservoir. The balloon was large enough to remain flaccid even when it occupied the entire reservoir volume. Prior to assembly of the reservoir, the balloon was tested for leaks by distending it with saline. After assembly, the saline remaining in the balloon was withdrawn through its connecting side arm so that no air remained trapped inside the balloon. The myocardium supplied by the distal LAD thus could be perfused with reservoir blood at the pressure transmitted to the outflow by the distention of the flaccid rubber balloon. Initially clamps A and B on the two side arms were closed and clamp C was opened, thus permitting normal perfusion of the artery. Immediately prior to the first injection of microspheres, the stopcock at the top of the reservoir was opened and the reservoir filled with arterial blood by opening clamp B (Fig. 1). The stopcock then was closed. Clamp A then was opened and clamp C closed, causing the empty balloon in the closed chamber to fill and displace reservoir blood into the distal LAD. During the time of distal LAD perfusion through the bypass system, as well as during perfusion from the reservoir, the perfusion pressure was monitored through a side arm at the level of the inserted cannula (not shown in Fig. 1). This pressure was the same under both conditions of perfusion and corresponded closely to aortic pressure (Fig. 2). Mean perfusion pressure was within 2 mm Hg of aortic root pressure, whereas phasic pressures transmitted through the carotid artery and the bypass system were similar to the distal aortic pressures. During diastole the aortic root pressure exceeded perfusion pressure by only 5-10 mm Hg and then only during a portion of diastole. Perfusion from the reservoir could be continued without reduction in pressure for at least 2.5 minutes. Thus all coronary arteries were perfused at similar pressures during perfusion through the bypass system from the carotid artery as well as from the reservoir, and blood flow in the interarterial anastomoses should have been minimal.

While the distal portion of the LAD was being perfused from the reservoir, the first set of microspheres was injected into the left atrium. While the microspheres destined for the LAD were trapped in the balloon, the myocardium perfused by vessels other than the distal LAD was uniformly marked with these microspheres. Thus this method permits accurate definition of the region normally supplied by the distal LAD. Myocardial samples obtained from the LAD region which were found to contain some microspheres from this first injection must have been composed of tissue that was normally perfused by blood delivered through unoccluded adjacent arteries as well as tissue normally supplied by the LAD. Following LAD occlusion, flow in the portion of the tissue perfused by other arteries continued to supply this tissue adequately. If blood flow in this normal tissue is equal to that in more distal samples from the myocardium entirely perfused by the circumflex artery, the amount of normal tissue "contaminating" samples from the LAD region can be estimated from the first set of microspheres. Therefore, the flow in the "contaminated" samples from the LAD region was equated to flow per unit tissue in the corresponding normal sample from the free wall of the left ventricle. In this way, the fraction of "contaminating" normal tissue included within each sample from the LAD region can be calculated. The absolute mass of normal tissue and, hence, the weight of the ischemic tissue alone could also be determined.

Two minutes after the injection of this first set of
FIGURE 2 Pressure characteristics recorded simultaneously during bypass and balloon perfusion. The tracings show from top to bottom the aortic pressure (AoP), the distal left descending coronary artery (LAD) perfusion pressure, and the left ventricular end diastolic pressure (LVEDP). After cannulation of the LAD, perfusion was continued through the bypass system from the left carotid artery. Mean perfusion pressure was nearly identical to aortic pressure, while phasic pressure transmitted through the carotid artery and the bypass system were similar to distal aortic pressures. During the injection of microspheres, perfusion was continued from the previously filled reservoir without any alterations in the characteristics of the perfusion pressure. To mark the duration of the injection of microspheres into the left atrium, the LVEDP was turned off. When the balloon in the reservoir was completely filled perfusion of the distal LAD ceased, causing the abrupt drop in the intravascular pressure to the peripheral coronary arterial pressure remaining in this portion of the vessel.

microspheres, the LAD was occluded by clamping the bypass system between the reservoir and the inserted cannula. Ten minutes later a second set of microspheres was injected. These microspheres now could be carried into the ischemic region only through preformed coronary collaterals and, hence, provided a measure of both the collateral blood flow in the ischemic tissue and myocardial blood flow in the normal tissue supplied by unoccluded vessels.

Following the second injection of microspheres, the atrial catheter and the cannula inserted into the LAD were removed. Fifty milligrams of protamine sulfate were administered and the pericardium and chest were closed. Air and fluid were evacuated from the chest cavity by a tube that provided suction for about 4 hours. Since systemic blood pressure was monitored during this entire period of early recovery, premature ventricular beats and tachyarrhythmias (which occurred in about one-third of these experiments) could be easily detected and successfully suppressed in every case by bolus injections of 40-80 mg of a 2% solution of lidocaine hydrochloride. The total dose of lidocaine hydrochloride administered to these dogs was about 120 mg. To balance fluid losses, the dogs received about 300 ml of saline. Furthermore, in each dog a single dose of sodium-ampicillin (500 mg) was administered, intramuscularly, and morphine sulfate (10-15 mg) was given subcutaneously. Additional morphine was given as often as required to ensure the comfort of the animal. Usually only one additional 10-15 mg dose was needed and in no case did total dose exceed 50 mg/dog over the 24-hour postoperative period.

On the following day, dogs were reanesthetized with about one-half of the initial dose of sodium pentobarbital because irreversible cardiac arrest had been observed in an earlier series of dogs undergoing the same experimental procedure when the full dose of pentobarbital was employed. The chest was then reopened and catheters again were inserted into the aorta and the left atrium. The dogs were again anticoagulated with heparin (5000 units, iv), and 24 hours after occlusion, a third set of microspheres was injected. This was followed by the hemodynamic measurements. The LAD was then recannulated at the site of ligation and 5-8 ml of a 2% solution of Evans blue dye were slowly injected by hand through the inserted cannula to stain the region myocardium originally perfused by the distal portion of the LAD. Immediately after the injection of the dye, the heart was excised and frozen quickly in bath of dry ice and alcohol.

In 10 dogs the procedure used initially to delineate the tissue supplied by the distal portion of the LAD was repeated 24 hours after occlusion and 24 hours after the injection of the dye to provide some insight into whether the tissue mass perfused by the occluded vessel had changed in size during the evolution of infarction.

**ADMINISTRATION OF MICROSPHERES**

Standard carbonized microspheres of 15 ± 5μm in diameter, labeled with the nuclides 141Ce, 85Sr, 51Cr, and 95Nb (3M Co.) were used to measure regional myocardial blood flow and cardiac output. A few drops of Tween 80 were added to the microspheres which then were suspended in a 63% sucrose solution. Prior to injection,
they were dispersed by mechanical agitation and sonication for 5 minutes in an ultrasonic bath. Ten ml of the solution containing 10⁶ beads were injected over a period of 20-25 seconds through the left atrial cannula which was subsequently flushed with 10 ml of saline. Usually no changes in the recorded hemodynamic parameters could be observed following the injection, but occasionally a decrease in aortic pressure of 10-20 mm Hg and lasting about 10-15 seconds was noted. Arterial blood was withdrawn starting just prior to the administration of microspheres and continuing for 30 seconds beyond the end of the injection using a Harvard pump with a withdrawal rate of 11.6 ml/min (Harvard Apparatus). This permitted the calculation of absolute tissue flows¹⁷ and cardiac output.¹⁸

TISSUE SEPARATION AND ANALYSIS

The frozen hearts were cut into 4-mm thick slices from the apex to the base with an electric slicing machine (Fig. 1). The region supplied by the distal LAD was clearly delineated from the normal myocardium perfused by unoccluded vessels by the Evans blue dye injected into the distal portion of the LAD. The blue dye failed to stain the subendocardial region with regularity and it appeared more or less homogenously white and often was surrounded by a thin rim of hemorrhagic tissue. This grossly necrotic tissue sometimes extended laterally into the normal myocardium without being separated from it by a blue-colored rim. The blue-stained tissue plus the central white zone comprised the total of the involved tissue. The stained and grossly necrotic tissue was separated from the unstained normal myocardium by careful dissection along the blue border. However, stained tissue extending into the free wall of the right ventricle was discarded. The natural bias in dissecting the blue-stained tissue from the adjacent normal unstained myocardium along the highly convoluted border tended to minimize stained tissue in the unstained border. Thus, inclusions of small unstained tissue portions in the border of the stained and grossly necrotic tissue could not be avoided and such inclusion could often be seen, but none of this tissue was discarded. The total weight of the stained and grossly necrotic tissue averaged 25.8 ± 1.2 g for the 53 hearts. From the apparent center of the stained region, a transmural sample was obtained and assayed separately. These tissue samples as well as a sample of normally perfused tissue from the posterior base of the left ventricle were kept frozen until they were assessed for their content of CPK activity, which was chosen as a marker of cell viability (see below).

In 24 hearts, the tissue between the sample from the apparent center of the stained region and the border towards the free wall of the left ventricle was considered as the "border ischemic" sample and processed separately. A sample of similar size was cut from the immediately adjacent normal tissue and was called the "border normal" sample. Thus, the sample from the center of the stained region, the border ischemic, and the border normal sample were continuous with each other and no tissue was discarded at the boundaries between the samples. Each sample had a width on the epicardial surface of about 5 mm. All these samples were divided into endocardial, midmyocardial, and epicardial segments, and the corresponding segments from each slice were pooled. However, no individual samples were taken from the apical slice and the slice nearest to the base, which showed only partial staining with the blue dye. The weight of the pooled tissue segments from the stained region averaged 1.03 ± 0.15 g and, from the normal myocardium, 1.13 ± 0.12 g.

CPK ACTIVITY

CPK was analyzed in all samples by the method of Kjekshus and Sobel.¹⁰ The myocardial samples were weighed, minced with scissors, and homogenized in 20 vol/g of iced 0.25 m sucrose, 0.001 m neutralized EDTA and 0.1 m mercaptoethanol in a Virtis "45" homogenizer (Virtis Co.) with two bursts of 15 seconds duration at a speed setting of 4. The homogenates then were centrifuged at 17,300 g for 20 minutes. The supernatant fractions were diluted 1:200 in a buffer containing 0.2% bovine albumin and 0.01 m Tris base (Trizma; Sigma), pH 7.4. The CPK activity was measured spectrophotometrically at 30°C using Calbiochem's CPK reagents, based on the methods of Oliver¹⁹ and Rosalki²⁰. The CPK activity was expressed in international units (IU) per gram wet tissue weight or in percent of CPK activity found in the control samples of the normal myocardium taken from the posterior base of the left ventricle. The determination of the CPK activity was not influenced by the admixture of Evans blue dye in the samples from the ischemic myocardium. The blue dye in the ischemic samples, however, interfered with the protein determination in the supernatant by the biuret method. In the control samples, the CPK activity related to the protein content was in each case quite similar to the values reported recently by others²¹ and averaged 45.1 ± 2.0 IU/mg protein.

REGIONAL MYOCARDIAL BLOOD FLOW

Because the microspheres were completely recovered in the pellet of the centrifuged homogenates, myocardial blood flows could be determined in the same sample in which the corresponding CPK levels were measured in the supernatant fraction. Therefore, all pellets of the tissue homogenates and the corresponding blood samples were subjected to γ-ray spectrometry using a Searle analytic model 1085 2-channel γ-ray counter with a 3-inch crystal (Searle Analytic). The number of microbeads present per gram of tissue averaged 507 ± 13 for the ¹⁴C-labeled microspheres, 635 ± 20 for the ⁸⁵Sr-labeled microspheres, 623 ± 22 for the ⁵¹Cr-labeled microspheres, and 590 ± 18 beads/g for the ⁹⁵Nb-labeled microspheres in the samples from the normal myocardium, whereas it was subsequently less in the samples from the ischemic or necrotic tissue. The data were corrected for background and crossover counts on a Wang Laboratories model 2200S computer, and the tissue flows and cardiac outputs were expressed in absolute units.
24 Hearts

TABLE 2

<table>
<thead>
<tr>
<th>Center ischemia</th>
<th>Border ischemia</th>
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<tbody>
<tr>
<td>Epicardium</td>
<td>2.3 ± 0.7</td>
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<tr>
<td>Midmyocardium</td>
<td>2.1 ± 0.7</td>
</tr>
<tr>
<td>Endocardium</td>
<td>2.9 ± 0.8</td>
</tr>
<tr>
<td>Transmurally</td>
<td>2.4 ± 0.6</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± 1 se.

STATISTICS

All results were expressed as mean ± 1 se. Paired comparisons with Student’s t-test were used to evaluate the statistical significance of differences in the data within and between the groups, and the regression lines were calculated by the least square fit method.

Results

The mortality rate due to arrhythmias or failure following coronary artery occlusion or resulting from surgery was 11.6% (7 of 60) during the time this study was conducted. Thus 53 of a total of 60 dogs recovered satisfactorily from the first intervention and were restudied successfully 24 hours after occlusion.

The general hemodynamic findings are summarized in Table 1. Although heart rate did not change over the 24-hour duration of the experiment, mean aortic pressure decreased significantly while mean left atrial pressure rose slightly over the same period of time. The cardiac output also decreased within the 24 hours. These data suggest a depression of cardiac function at restudy which may be explained by a combined effect of the surgical procedures and mild left ventricular failure due to myocardial infarction.

GROSS APPEARANCE OF THE INFARCTS

The myocardium supplied by the distal LAD was well delineated by the Evans blue dye injected at the end of the experiment. In each heart, however, the dye failed to penetrate the subendocardial layers and, to a various extent, the midwall and sometimes even the epicardial layers, which then appeared homogeneously white or, occasionally, slushy brown-grey. Since the absence of patent vessels apparently did not allow the blue dye to enter and thus stain this region, it was considered to be grossly necrotic; this conclusion was supported by histological examination.

This grossly necrotic tissue, which was often surrounded by a narrow hemorrhagic rim (<1 mm), occupied approximately the same thickness of wall up to the lateral borders of the lesion. Sometimes it was not separated from the adjacent normal myocardium by blue-stained tissue, as might have been expected, and in most of the cases only a very small blue rim of tissue was present between the lateral borders. In contrast to the grossly necrotic myocardium, the tissue overlaying the infarct was stained dark blue except for an occasional isolated focus. Although this stained tissue also was ischemic to some extent, it could still be perfused and thus might be capable of survival. Even though the stained and grossly necrotic tissue in general occupied a sector-shaped region of the anterior wall of the left ventricle, the lateral borders toward the normal unstained myocardium were highly irregular, and the epicardial landmarks could in no way be used to predict whether the underlying tissue belonged to the tissue supplied by the distal LAD or was normally perfused by adjacent vessels. Since it was not possible to cut the stained and grossly necrotic tissue precisely along the highly convoluted demarcation to assure that the tissue under study did not represent an admixture of ischemic and normal tissue, we used the technique described in Methods to define the tissue supplied by the distal portion of the LAD. By this technique of marking the normally perfused tissue prior to occlusion with microspheres while perfusing the distal portion of the LAD with unlabeled blood from the reservoir at aortic pressure, it was possible to delineate the tissue mass subsequently rendered ischemic. Following occlusion, flow in the tissue supplied by vessels other than the distal LAD remains and continues to supply the tissue adequately. Thus, the CPK activity in these inclusions also can be assumed to be the same as in the more distal normal myocardium. As was to be expected, the admixture of normally perfused tissue which was detected by the content of microspheres from the first injection was highest in the sample from the border of the stained and grossly necrotic region, averaging in the transmural sample 37.2 ± 3.6% of the tissue weight for all 24 hearts studied (Table 2). Since the natural bias in dissecting the tissue at the demarcation between stained and unstained myocardium was to include all of the stained tissue in this so-called border ischemic sample, some admixture of clearly unstained tissue in these samples could not be avoided.

It is of interest, however, that even in the samples from the center of the ischemic region we found small amounts of tissue normally perfused by unoccluded vessels. This admixture averaged 2.4 ± 0.6% of the tissue weight in the transmural sample from the center. Although not significant statistically, segments from the endocardium...
tended to have a somewhat higher admixture of tissue perfused by unoccluded vessels than the segments from the epicardium or from the midmyocardium. Transmural myocardial blood flow in the normal region during the first microsphere injection was 1.132 ± 0.050 ml/min · g⁻¹.

DIFFERENCES IN THE CPK ACTIVITY BETWEEN THE CENTER AND THE BORDER SAMPLE FROM THE STAINED REGION AND THE ADJACENT NORMAL MYOCARDIUM STUDIED IN 24 DOGS

Since the contamination with tissue normally supplied by unoccluded vessels is substantial, at least in the samples from the border of the stained region, there are significant differences in the uncorrected CPK activity between the samples from the center and the border of the ischemic region (Table 3, Fig. 3A). The CPK activity in the transmural sample from the adjacent normal myocardium did not differ from the CPK activity of the remote normal sample from the posterior base of the left ventricle which averaged 1750 ± 65 IU/g of tissue. No difference in CPK activity was found across the heart wall in the normal myocardium; this is in agreement with earlier observations.¹¹ Note again that the samples from the center and the border of the stained region, as well as the samples from the immediately adjacent unstained normal myocardium, were continuous with each other and that no tissue was discarded between the samples. Thus, if one disregards the admixture of normal tissue in the stained samples, a zone of the intermediate CPK depletion indeed seemed to exist and surround laterally the more depleted center zone.

However, when these data were corrected for the contamination by normal tissue inadvertently included in all these samples and especially in the ones obtained from the border of the stained region, as estimated from their content of microspheres from the first injection, the differences between the transmural samples as well as the individual segments from the center and the border of the ischemic region no longer existed (Table 3, Fig. 3B). There was now a sharp rise in CPK activity only at the demarcation between the tissue supplied by the distal portion of the LAD and the tissue supplied by unoccluded vessels. Within the region supplied by the distal LAD, the CPK depletion was uniform from the center to the edge. However, and despite the correction applied, a highly significant gradient in CPK activity remained across the heart wall. CPK activity was most reduced in the endocardium, averaging about 25% of the CPK activity in the normal myocardium, and was least reduced in the epicardium where it was about 40% of normal values (P < 0.001); this is consistent with the findings of Kjekshus and co-workers.²⁵

To confirm this result further, the CPK activities were compared in those samples from the center and the border of the stained region which had less than 10% contamination, as demonstrated by microspheres. A correction for the normal tissue included within these samples would be small and could be omitted. Eight pairs of corresponding center and border samples from the stained region of six hearts satisfied this criterion. The average contamination in the center samples was 3.8 ± 1.1% of the sample weight and in the border samples 4.8 ± 1.4%. We found no statistically significant difference in the CPK activity between these samples from the center and the border of the stained region. The CPK activity in the center averaged 872 ± 132 IU/g of tissue and 1045 ± 120 IU/g of tissue in the border (P = NS). In addition, the corresponding samples from the adjacent normal myocardium showed a CPK activity of 1627 ± 115 IU/g of tissue. This value was significantly different from the CPK activity of the border sample of the stained region (P < 0.001) but, otherwise, neither differed from the CPK activity of all samples from the border of the normal myocardium (P = NS) or from the CPK activity of all samples from the remote region of normal myocardium (P = NS). Thus, the possibility was excluded that in obtaining these samples the dissection was performed within the ischemic region rather than at the border between the ischemic and the normal region.

If this reasoning is correct, the gradient of the CPK activity between the border and the center samples of the stained region should be proportional to the fraction of the normal tissue included within the border samples. Indeed, a significant relationship between these two parameters was found in the 87 observations (P < 0.001), indicating that the increasing contamination with normal tissue of the border samples from the stained region is reflected by a proportional increase in the CPK activity of

### Table 3

<table>
<thead>
<tr>
<th></th>
<th>Uncorrected values</th>
<th>CPK activity (IU/g tissue)</th>
<th>Values corrected for included normal tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Center ischemia</td>
<td>Border ischemia</td>
<td>Border normal</td>
</tr>
<tr>
<td>Epicardium</td>
<td>950 ± 67</td>
<td>1280 ± 92</td>
<td>1850 ± 150</td>
</tr>
<tr>
<td>Midmyocardium</td>
<td>670 ± 50</td>
<td>1105 ± 66</td>
<td>1830 ± 130</td>
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<tr>
<td>Endocardium</td>
<td>480 ± 40</td>
<td>1070 ± 65</td>
<td>1800 ± 150</td>
</tr>
<tr>
<td>Transmurally</td>
<td>700 ± 49</td>
<td>1160 ± 66</td>
<td>1830 ± 140</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± 1 se. Statistical analysis was performed by paired t-tests.

* NS = not significant.

²⁵ Values corrected for inadvertently included normal tissue within the samples from the ischemia of 24 hearts.
these samples. For each set of values, the difference in CPK activity between border and center samples from the stained region was normalized by dividing it by the CPK activity difference between the normal and center sample. The normalized CPK gradient (y) was then plotted against the fraction of normal tissue included in the border sample from the stained region (x). Based on our theory, a slope of unity is expected for the line fitted to this plot. However, slope of 0.67 provided the best fit: y = 0.67x + 0.055. This suggests that the border samples might even have been more depleted of CPK than the samples from the center of the ischemic region.

**CPK ACTIVITY IN THE TRANSMURAL SAMPLE FROM THE CENTER OF THE ISCHEMIC REGION VS. CPK ACTIVITY OF THE ENTIRE ISCHEMIC MYOCARDIUM**

Figure 4 shows the comparison between the CPK activity left in the transmural sample from the center of the ischemic region and the CPK activity determined in the entire ischemic myocardium for all 53 dogs studied. The mean values corrected for contamination with normal tissue as detected by the method mentioned are: 34.7 ± 2.1% of the CPK activity in normal myocardium for the transmural sample from the center of the ischemic region and 39.0 ± 2.2% for the entire ischemic region (P < 0.01). The slight differences in the two mean values can be accounted for, in part, by the difference between the cylindrical shape of the transmural sample from the center and the shape of the entire ischemic mass. The sample from the center was composed of almost equivalent masses of endocardial and epicardial tissue halves: 1.57 ± 0.13 g vs. 1.61 ± 0.14 g, respectively (P = NS). In contrast, the cross-section of the entire ischemic mass approximated the portion of a sector between the outer and inner radii of the ventricle and thus was composed of relatively more epicardial tissue than endocardial tissue. Since the CPK is less depleted in the epicardial layers than in the endocardial ones, there is a slightly higher CPK activity in the entire ischemic region, compared with the CPK activity in the transmural sample from the center of the ischemic region.
The epicardial and endocardial halves of the stained and grossly necrotic tissue were estimated by comparing the areas occupied by these halves in all the slices of five hearts in order to correct for the disproportion in these relative volumes. The endocardial half comprised 39.7 ± 1.0% and the epicardial half comprised 60.3 ± 1.0% of the totally stained and grossly necrotic tissue. The relative masses occupied by the endo- and epicardial halves can also be calculated for a spherical model of the ventricle. Based on our own observations, as well as those reported by others,24-25 a volume of 100 ml and a wall thickness of 13 mm were assumed. The endocardial half would then comprise 35.2% and the epicardial half 64.8% of the total wall of the sphere. Using the values obtained from the five hearts studied for this purpose and the differences in the CPK activity found between the endo- and epicardial halves in the samples of 28 animals, which averaged, respectively, 27.6 ± 2.9% and 48.0 ± 4.0% of the CPK activity in normal myocardium (P < 0.001), values for the transmural sample from the center of the ischemic region could be appropriately corrected. The mean CPK activity left in the transmural sample from the center of the ischemic region thus adjusted for geometrical factors amounted to 36.6 ± 2.2% of the CPK activity in normal myocardium. This was not statistically different from the CPK activity in the entire ischemic region, which averaged 39.0 ± 2.2%. These results again suggest that the CPK is uniformly depleted throughout the entire ischemic region.

TISSUE MASS SUPPLIED BY THE DISTAL PORTION OF THE LAD BEFORE AND 24 HOURS AFTER OCCLUSION

It could be argued that the technique used to delineate the region perfused exclusively by the distal portion of the LAD before occlusion is performed would not account for the tissue mass in the vascular network lying between the LAD and the left circumflex (LCF) arteries, where a substantial dual circulation may exist. When a LAD occlusion is performed, a border zone of intermediate low levels might then emerge in this tissue which was previously supplied from both the LAD and the LCF arteries, due to the large drop in perfusion pressure in the LAD. With time, perfusion of this tissue from the adjacent unoccluded vessels would increase. Closer to the LAD, necrosis might develop and lead to an interruption in vascular continuity between the two supply zones. While the large vessels may remain perfusable, perfusion defects develop in the small coronary vessels as early as 90 minutes after occlusion.26 Reperfusing the distal portions of the LAD with blood at aortic pressure from a reservoir while injecting microspheres 24 hours after occlusion would certainly prevent the microspheres from entering the distal LAD region through the collateral vessels. However, this would not affect the distribution of these microspheres in the tissue that had previously been supplied by both the LAD and the LCF but had become entirely supplied by the LCF once the perfusion defects in the small vessels of the distal LAD had developed. Thus, the tissue mass delineated prior to occlusion by labeling the normal myocardium with microspheres would tend to overestimate the tissue actually involved. We therefore labeled the normal myocardium with microspheres in 10 dogs before and 24 hours after occlusion as described above. The calculated mass of tissue that belonged to the region of perfusion of the distal LAD was nearly identical before and 24 hours after occlusion and averaged 27.4 ± 3.0 g and 26.5 ± 4.1 g, respectively (P = NS) (Fig. 5). This strongly suggests that the normal and the ischemic tissue comprise two separate regions with a discrete anatomical and functional boundary between them. Any dual circulation that may exist between the different regions under normal circumstances appears to be of little importance when occlusion of a coronary artery reduces the blood flow in one of the regions.

CPK ACTIVITY DETERMINED 24 HOURS AFTER OCCLUSION VS. COLLATERAL BLOOD FLOW MEASURED 10 MINUTES AFTER OCCLUSION

If blood flow is a critical determinant of the survival of tissue, the residual collateral blood flow in the ischemic region should be related to the ultimate amount of necrosis. The transmural CPK activity remaining in the center of the ischemic region 24 hours after occlusion was therefore compared with the collateral blood flow determined 10 minutes following occlusion in 28 dogs. A close relationship was obtained (r = 0.90) (Fig. 6). In addition, the highly significant difference in the CPK activity between the endocardial and epicardial halves of each transmural sample averaging, respectively, 27.6 ± 2.9% and 48.0 ± 4.0% of the CPK activity in normal myocardium, (P < 0.001), was also paralleled by a similar difference in the collateral blood flow to these samples as measured 10 minutes after occlusion. It averaged 0.107 ± 0.026

![Figure 5](image-url)
vascular beds of different regions interdigitate to a various extent, correct identification of these regions of tissue is the precise delineation of the tissue at risk. Because sis.

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initial residual perfusion of the ischemic region is a major

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close relation exists between the initial level of colateral

blood flow and subsequent CPK depletion 24 hours after

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occlusion in the transmural tissue sample from the center of the ischemic region as well as in the samples from the endocardial and epicardial halves. This suggests that the initial residual perfusion of the ischemic region is a major determinant of the subsequent extent of myocardial necro-

sis.

A major consideration in these observations concerns the precise delineation of the tissue at risk. Because vascular beds of different regions interdigitate to a various extent, correct identification of these regions of tissue is imperative. In this study, these regions have been shown grossly by the injection of blue dye into the occluded vessel distal to the site of ligation and more precisely by the labeling of the normal myocardium with microspheres. Although tissue samples from the geometric center of the infarct are likely to contain little or no contaminating normal tissue, substantial admixture of normal tissue in samples cut from the lateral borders between the tissue at risk and the normal myocardium will necessarily result in intermediate values, compared with the center of the ischemic region. Thus a border zone of intermediate levels of flow or of only moderately depressed mechanical or biochemical properties would be created by improper sampling of the tissue.

Furthermore, changes in regional flow or in the me-

chano- or electrophysiological behavior occurring in this lateral "border zone" may be caused by corresponding changes within the normal tissue contaminating this region. Thus, such changes may reflect the alterations in the normal myocardium rather than in the ischemic tissue. This emphasizes further the necessity to define clearly the tissue under study; this is particularly important in relation to current studies on salvaging ischemic myocardium by a great variety of proposed interventions.27

Our observations appear to conflict with those of an earlier histopathological study26 which identified areas of ischemia and moderate tissue damage surrounding the central infarct. Whereas the central zone exhibited complete architectural destruction, this "border zone" appeared undisturbed except for swollen mitochondria of

Discussion

The results of the present study demonstrate that 24 hours following occlusion of the LAD in the dog: (1) creatine phosphokinase is uniformly depleted from the lateral edge to the center of the myocardial region supplied by the occluded LAD and no transitional zone of intermediate CPK depletion separates the center of the infarct from the laterally adjacent normal myocardium; (2) the endocardial layers are more depleted of CPK after 24 hours than the epicardial layers; (3) the tissue mass perfused by the LAD represents functionally a separate entity but interdigitates anatomically to a various extent with the tissue perfused by other vessels; and (4) a
close relation exists between the initial level of collateral blood flow and subsequent CPK depletion 24 hours after occlusion in the transmural tissue sample from the center of the ischemic region as well as in the samples from the endocardial and epicardial halves. This suggests that the initial residual perfusion of the ischemic region is a major determinant of the subsequent extent of myocardial necro-

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twice normal in size, swelling of the A-bands, and accumulation of neutral fat droplets. Swollen mitochondria were identified after the specific staining of the tissue for dehydrogenase enzymes, since then they could be detected as heavily stained black dots with moderate magnification and without the need for oil immersion. In contrast, oil immersion had to be used to visualize the mitochondria in the more remote normal tissue which also were stained but to a lesser extent. These results are contrary to the observations of Lushnikov\textsuperscript{29} and Vikhert and Cherpakenko\textsuperscript{29} who used similar methods and found decreased dehydrogenase activities outside of the clearly infarcted tissue. The changes observed in the mitochondria from the "ischemic" area require more precise definition since swelling of these structures has been related by others to irreversible damage.\textsuperscript{30} Moreover, in Cox's study,\textsuperscript{12} the area defined as ischemic decreased significantly in size with time whereas the necrotic area did not change within the first 7 days after coronary artery occlusion; this suggests that the actual size of the infarct is determined early after occlusion and casts some doubt on the true nature of the zone surrounding the central necrosis.

The existence of a very thin rim of moderately damaged tissue measuring 1 mm or less in width within the zone of demarcation between the ischemic and the normal myocardium cannot be entirely excluded on the basis of our data. The results presented in this study, however, strongly indicate that no large amounts of tissue with intermediate flow and, consequently, reduced tissue damage surround laterally a more deeply ischemic and finally grossly necrotic central zone. Thus, small vessel connections at the lateral edge apparently do not create a noteworthy transitional zone between normal and ischemic tissue, and an existing dual circulation in the region of demarcation does not seem to be of significance in an acutely ischemic situation. Under conditions of chronic ischemia, however, these vascular connections at the lateral borders might well become important. In addition, our conclusions are limited by the use of CPK depletion to indicate cellular necrosis. However, a large body of evidence has supported this association.\textsuperscript{10} Furthermore, our measurements, which were made 24 hours after coronary artery occlusion, may not indicate the status of the myocardium at other periods of time.

Differences within the ischemic region, however, do exist across the heart wall and one is tempted to call the epicardial layers the "border zone" of the infarct, since they have the highest flow levels immediately after and the least CPK depletion 24 hours after occlusion. This may be due to hemodynamic factors within the ischemic region which result in a lower perfusion of the subendocardium. However, aggravating this situation may be other factors such as increased energy requirements due to a higher basal metabolism in the deep layers.\textsuperscript{21} As infarction continues, vasculature in the subendocardium also becomes necrotic and this leads to a further redistribution of flow within the ischemic region from the endocardium to the epicardium.\textsuperscript{22} Nevertheless, the epicardial layers do not represent a transitional zone between the deeply ischemic subendocardium and the normal tissue.

Even if they are less ischemic than the subendocardial layers, they are distinctively separated from the surrounding normal tissue and their perfusion depends entirely on the blood supply delivered to the ischemic region through the collateral vessels and the blood distribution within the ischemic region.

In view of these findings, studies of drugs effective on ischemic myocardium will have to localize the tissue to be studied relative to the ischemic border and define its position across the heart wall. However, since the center of the ischemic and infarcted region appears to be representative of the entire tissue mass involved in the process of ischemia, a single transmural biopsy from the center of the region will be sufficient to characterize the situation in the tissue at risk.

The results presented in this study indicate further that the coronary arterial tree is functionally an end-artery system. The flow in the ischemic tissue is delivered to this region through preformed interarterial anastomoses, i.e., the collateral vessels, and is distributed to the tissue through the original vascular tree distal to the site of occlusion. The lateral limits of the ischemic region is thus determined by the region of distribution of the arterial bed while the extension of the necrosis across the heart wall depends largely on the status of perfusion within the ischemic region. Histopathological evidence also suggests that necrosis does not extend as time progressively.\textsuperscript{12} Extension of an infarct, should it occur, has to be explained by other mechanisms such as additional vascular obstruction in adjacent vascular systems which were not involved in the first occlusion or relative ischemia in the normal tissue surrounding the ischemic tissue due to increased wall stress at the demarcation between contracting and noncontracting tissues, or by interruption of vessels supplying large interdigitations of normal tissue within the originally ischemic tissue due to changes associated with the process of infarction in the ischemic tissue such as hemorrhage or edema. Alternatively, much that is called extension of infarction may only be involving more of the wall transmurally without lateral extension.

Attempts to correlate the depletion of CPK of the tissue with the flow in the tissue at the end of the experiment have been made earlier.\textsuperscript{13} The good relationship between flow measured immediately after occlusion and the CPK depletion 24 hours later, however, emphasizes that the tissue flow existing early after occlusion is the primary determinant of the ultimately emerging infarct size, since it has been shown\textsuperscript{29} that at later stages, i.e., 48 hours after occlusion, the degree of CPK depletion is not significantly different from the depletion at 24 hours. Moreover, this finding suggests that the early collateral blood flow within the acutely ischemic tissue can be used to predict resulting necrosis. It has to be pointed out, however, that besides the collateral blood flow within the tissue, other factors may influence the degree to which the necrosis extends transmurally.\textsuperscript{22}

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