The Histological Lateral Border of Acute Canine Myocardial Infarction
A Function of Microcirculation

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SUMMARY Studies from this laboratory have shown that the border of a 24-hour canine infarct is histologically sharp and is composed of numerous interdigitating peninsulas of necrotic and normal tissue. To see if this sharp boundary is spatially related to the capillary beds of occluded and non-occluded arteries, the left anterior descending artery (LAD) was ligated in five mongrel dogs. Twenty-four hours later, white silicone rubber (Microfil) was injected into the LAD distal to the ligature; simultaneously and under the same pressure, red Microfil was injected into the left main coronary artery (LMCA). In hematoxylin and eosin sections from the border of the infarct, capillaries supplied by the LAD (white) were either in areas of necrosis, in normal epicardium or, rarely, in normal tissue along the lateral boundary; those supplied by the LMCA (red) were almost always in normal regions. Quantitative evaluation of this relationship revealed that the majority of the vessels in the normal and necrotic tissue were concordant (i.e., that normal tissue was supplied by the LMCA, and necrotic tissue by the LAD). However, a small zone of vascular discordance, averaging approximately 30 μm in width, was present along the infarct boundary, possibly representing a narrow border zone of little consequence. Hence, the complex interdigitation of normal and necrotic tissue in the lateral border of an infarct is predominantly a function of the interdigitation of the capillary beds supplied by the occluded and nonoccluded arteries.

THREE-DIMENSIONAL reconstructions of fully developed acute canine myocardial infarctions have revealed an extremely irregular but sharp boundary demarcating necrotic and normal myocardium (Factor et al., 1978). Isolated islands of normal myocardium, thought by some to represent a border zone of ischemic but viable tissue (Edwards, 1957; Braunwald et al., 1974), were shown to be peninsulas of myocardium in continuity with the main mass of normal heart muscle (Factor et al., 1978). Several recent studies, employing different analytical techniques, also have confirmed that when myocardial infarctions are analyzed spatially, the boundary between necrotic and normal tissue is remarkably discrete, with no obvious border zone present (Mar-
scribes an analysis by serial section of acute, fully developed 24-hour myocardial infarction in which both the occluded and non-occluded coronary vasculature is identified at the tissue level and correlated with the presence or absence of cellular necrosis. In these studies, the vascular and lateral histological borders are shown to be discrete, with minimal overlap, thus supporting the concept that the vascular anatomy determines the geographic pattern of the fully developed infarct.

Methods

Five mongrel dogs weighing from 19 to 27 kg were anesthetized with sodium pentobarbital (30 mg/kg). A polyvinyl catheter was placed in the femoral vein for administration of fluids and drugs if needed; a similar catheter was placed in the femoral artery for measurement of aortic pressure. The dogs were intubated with a cuffed endotracheal tube and ventilated with 100% oxygen. Under aseptic conditions, a left thoracotomy was performed in the 5th intercostal space. The heart was exposed and the left anterior descending coronary artery (LAD) isolated and acutely ligated below its first diagonal branch. The chest then was closed and the fluid within the thorax was evacuated by suction for several hours until artificial ventilation was discontinued. Morphine sulfate, 10 mg, was administered intramuscularly.

The dogs were studied 24 hours after coronary artery occlusion. The animals were anesthetized again and catheters were placed in the femoral artery and vein. A tracheostomy was performed and the subclavian artery was isolated near its point of origin. A large-bore cannula was inserted into the left main coronary artery (LMCA) via the subclavian. Another cannula was inserted into the LAD just distal to the ligature.

White Microfil, a silicone rubber compound (Canton Bio-Medical Products, Inc.) that fills the entire vasculature, including vessels of capillary size, was injected into the LAD, and red Microfil into the LMCA. Both injections were done simultaneously and under the same pressure by connecting a pressure bottle to two 20-ml syringes in parallel, which served as reservoirs for the Microfil. These reservoirs were connected to the cannulae via polyvinyl tubing that was clamped with hemostats. Nineteen ml of each color of Microfil were prepared immediately prior to the injection procedure. The injection was performed in the following manner. The hemostats were released simultaneously and the pressure brought from 0 mm Hg up to 100 mm Hg. When the syringe containing the red Microfil was almost empty (the red always emptied first because the LMCA supplies a larger area than the LAD), the pressure was dropped to 0 mm Hg and both hemostats were clamped.

The hearts were excised and placed in ice for 3 hours to "cure" the Microfil; this was followed by fixation in 4% buffered formaldehyde. The fixed hearts then were sliced by hand into approximately five rings, from apex to base, with slices made distal to the LAD ligature. We removed a sector of the tissue ring which extended from the center of the visually identified infarct to the surrounding normal myocardium. This tissue included the interface between the red- and white-filled capillary networks. The sample of myocardium was cut into 8 to 12 0.5-cm sections and each section was embedded in paraffin. The orientation of the sections with respect to epicardium and endocardium and to the base to apex axis was noted carefully. The sections were numbered sequentially so that it was possible to identify the infarct zone (filled with white Microfil), the capillary interface ("the border zone"), and the normal zone (filled with red Microfil). Six-micron histological sections of the tissue were stained with hematoxylin and eosin. The histological criteria used for the evaluation of tissue necrosis were the same as those described previously (Mallory et al., 1939; Blumgart et al., 1941; Factor et al., 1978). Blocks of myocardium that revealed a transition between necrotic and normal tissue (the lateral border zone) were completely sectioned at 200-μm intervals, so that the geographic features of this area could be traced sequentially and in their entirety. Approximately 40–70 such serial sections were prepared from each heart.

Routine transillumination of the tissue sections revealed that the Microfil-perfused vessels, whether red or white, appeared black because the opaque silicone rubber absorbed light. Therefore, it was necessary to employ epi-illumination to identify the color of the perfusate. Sections were photographed with transillumination and then the same field was epi-illuminated and photographed. This technique allowed us to reconstruct visually the progression of tissue peninsulas at the border of the infarct, as well as to identify the origin of the vessels supplying that region.

Matched trans- and epi-illuminated transparencies from each dog were selected if they revealed maximal interdigitation of normal and necrotic myocardium, and if they demonstrated adequate capillary perfusion with Microfil in both areas. A total of 10 such pairs was evaluated. The transparencies were projected onto white paper at a fixed magnification (250×), so that the normal and infarcted tissue outlines could be traced. The matched epi-illuminated slide then was superimposed on the tracing and all white and red silicone rubber-filled capillaries were identified and marked on the paper. Any white-filled (LAD) capillaries present in tissue identified as normal, and any red-filled (LMCA) capillaries in tissue identified as necrotic, were considered to be discordant. The distance of these vessels from the nearest concordant border was measured on the tracing and calculated in microns. Vessels present on the traced line at the border were counted as concordant.
Results

In the five animals studied 24 hours after occlusion of the LAD, sharp demarcation was observed between groups of hypereosinophilic, hyalinized necrotic cells and normal myocardium (Figs. 1a and 2a). Necrosis was homogeneous in the subendocardial portion of the sections, whereas it was variable in the epicardial regions. Marked interdigitation between normal and necrotic tissue peninsulas was present along the lateral border of the infarct. As previously described (Factor et al., 1978), these

Figure 1  a: There is a sharp localization between the normal myocardium in the upper portion of the field and the hypereosinophilic infarcted myocardium below, delimited by the thick arrows. In the lower portion of the field, there is homogeneous necrosis, whereas at the border there is an admixture of normal and necrotic tissue, with representative normal cells marked by the thin arrows. Several masses of extravasated Microfil are present within the infarct zone. Note that the Microfil appears black when viewed with trans-illumination (185x). b: The same field in part a, viewed with epi-illumination, reveals that the normal myocardium is supplied by red Microfil-perfused vessels derived from the LMCA, whereas the necrotic myocardium is supplied by white Microfil-perfused vessels derived from the ligated LAD. The diminished density of capillary filling in the necrotic tissue can be appreciated easily in this field. Several areas of clearly infarcted myocardium observed in part a seem to have no capillary perfusion in this part and therefore appear as blank areas. In the zone immediately subjacent to the thick arrows in which there is an admixture of normal and necrotic cells, there is no admixture of red- and white-filled capillaries. The normal cells marked by the thin arrows are spatially related to individual LMCA red-filled vessels. A larger vessel in the infarction zone appears to be filled with both red and white Microfil. Such observations were distinctly rare, and probably represent filling of small veins draining perfusion fields. (185x).

Figure 2  a: Hypereosinophilic, necrotic myocardium is clearly distinguished from the normal myocardium above the thick arrows. Within the normal zone there is an admixture of necrotic cells, with two such individual cells marked by arrowheads. A peninsula of normal myocardium (thin arrow) extends into the infarct zone (185x). b: Epi-illumination of the field observed in part a reveals that the infarcted myocardium is supplied by vessels derived from the LAD, whereas the normal myocardium is supplied by the LMCA. Variations in capillary density between the normal and necrotic tissue can be appreciated easily. The single necrotic cells marked by arrowheads are spatially related to white Microfil-perfused myocardium. Similarly, the normal peninsula of myocardium (thin arrow), though surrounded by necrotic myocardium, is supplied by LMCA capillaries. The sharp histological separation between abutting normal and necrotic cells is matched by the close apposition of LMCA and LAD capillaries, which in some areas (far right arrowhead) appear to be separated by the diameter of a single cell (185x).
peninsulas could be traced back to confluent masses of homogeneous normal or necrotic myocardium. In some areas, there was pronounced intermingling of small numbers of infarcted (or normal) cells within an expanse of the other tissue.

When the histological sections were viewed with trans-illumination, the Microfil-containing vessels appeared black (Figs. 1a and 2a). Two differences between vessels in the normal and infarcted myocardium were noted. The density of capillary filling in the infarct zone was frequently less than the capillary filling in the normal tissue (Figs. 1a, b; 2a, b) presumably due to the "no-reflow" phenomenon (Koner et al., 1974). In the quantitative study (Table I), an average of approximately 100 fewer capillaries was present in the necrotic zone, when compared to the normal zone. Capillaries in the normal myocardium appeared to be filled almost completely with the silicone rubber compound, although they did not all "light-up" when photographed by epi-illumination. Additionally, focal extravasation of Microfil was apparent in the infarct zone, due to vascular disruption during the pressure injection. The extent of extravasation varied considerably from animal to animal.

Consecutively viewed trans- and epi-illuminated sections revealed a sharp vascular demarcation between the necrotic and normal myocardial peninsulas (Figs. 1-4). Infarcted myocardium, whether within a confluent mass of necrotic tissue, or seemingly isolated and surrounded by normal tissue, generally had capillaries filled with white Microfil (derived from the occluded LAD). This observation required frequent switching between epi- and trans-illumination because some necrotic tissue had only minimal capillary filling, and often appeared as unperfused regions when viewed with epi-illumination alone. Normal isolated or confluent myocardium was supplied predominantly by red-filled capillaries (derived from the LMCA). The vascular border was as sharp and as easily identified as the histological border. Extensive overlapping of the two separate circulations was not observed along the lateral border of the infarctions; i.e., we did not find large numbers of LAD capillaries (white) in normal myocardial peninsulas, nor did we find numerous LMCA capillaries (red) in completely necrotic peninsulas (see Table 1). In the epicardial myocardium, however, we did find some zones of normal muscle supplied by white and white-red double-filled vessels. These double-filled vessels were perfused, presumably from both the LAD and LMCA, and represent direct evidence for perfusion of the surviving subepicardial zone by a collateral circulation derived from the LMCA region. Double filling of subendocardial vessels was rare along the lateral border of the infarct.

In regions in which there was a pronounced admixture of normal and necrotic cells, we found a similar admixture of red- and white-filled capillaries (Fig. 2, a and b). Occasionally, we could identify single clusters of normal cells with red-filled capillaries in the immediate vicinity, while abutting necrotic cells were adjacent to white-filled vessels. Quantitative evaluation (Figs. 3 and 4; Table I) revealed approximately equal but small numbers of discordant vessels on either side of the complex infarct border. This discordance was present within a narrow zone, measuring an average 30 μm in width, closely hugging the border. In general, the discordant vessels represented a minority of the vessels within any one area. They also were not observed paralleling the border in a diffuse band, but rather were focal and irregular. Although we could not rule out the possibility that this narrow zone of discordance resulted from misinterpretation of normal or necrotic myocardial cells, or incorrect evaluation of Microfil color, it seems reasonable to conclude that, within the technical limitations of the technique, there may be a focal region 30 to 50 μm wide along the border with incongruous vascular supply. However, as a rule, it appears that the

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**Table 1** Quantitative Evaluation: Capillary Filling Vs. Infarct

<table>
<thead>
<tr>
<th>LAD (white)</th>
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<th>LMCA (red)</th>
<th></th>
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<tbody>
<tr>
<td>No. of vessels counted</td>
<td>No. of vessels discordant</td>
<td>Mean distance discordant (microns)</td>
<td>No. of vessels counted</td>
</tr>
<tr>
<td>1</td>
<td>107</td>
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</tr>
<tr>
<td>10</td>
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<td>17</td>
<td>30</td>
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**Mean ± SD**

<table>
<thead>
<tr>
<th>LAD (white)</th>
<th>LMCA (red)</th>
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<tbody>
<tr>
<td>175 ± 111</td>
<td>288 ± 109</td>
</tr>
<tr>
<td>23 ± 13</td>
<td>25 ± 17</td>
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<tr>
<td>33 ± 11</td>
<td>30 ± 10</td>
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</tbody>
</table>

**LAD =** left anterior descending coronary artery (occluded vessel); **LMCA =** left main coronary artery (unoccluded vessel). No. of vessels discordant: the number of epi-illuminated identifiable LAD (white) vessels outside of the infarcted tissue in the normal zone, or LMCA (red) vessels within the infarcted tissue. Mean distance discordant: the measured mean distance in microns of all discordant vessels from their nearest concordant border.
predominant determinant of tissue death or survival, even at the cellular-capillary level, specifically depends on whether an individual cell derives its nutrition from a capillary perfused by an occluded or patent artery.

**Discussion**

In a previous study (Factor et al., 1978), we employed a serial section technique to reconstruct the histological border zone of fully developed (24-hour) canine myocardial infarctions. We demonstrated that the lateral border region was composed of a complex but discrete interdigitation of normal and necrotic tissue peninsulas, which extended as finger-like projections from the main masses of uninvolved or infarcted myocardium. Based on histological criteria of acute necrosis, we did not observe isolated groups or islands of cells surviving in a "twilight zone" (Edwards, 1957), corresponding to a zone of intermediate ischemia. The principal observation of the present report is that the histological extent and interdigitation of normal and infarcted myocardium is a function of the interdigitation of the capillary beds supplied by the occluded and non-occluded arteries.

**Physiology and Anatomy of the Lateral Border Zone**

A major question posed by the first study was the physiological basis for maintaining the viability of seemingly normal tissue peninsulas, even when they were surrounded completely by necrotic myocardium. Was their viability a result of microvascular supply from the unoccluded large epicardial coronary artery, or was it the consequence of extensive collateral networks at the tissue level between the occluded and the patent coronary vessels? If the former pattern was present, then a sharp histological border could be explained by necrosis of only the tissue at risk (i.e., that supplied by the occluded vessel). If the latter arrangement was present, then, despite the histological appearance of seeming viability, the tissue peninsulas might very well be ischemic but surviving in a border zone interposed between completely normal and necrotic myocardium.

To the best of our knowledge, the present study is the first to examine this question specifically by correlating the microvascular supply with the histological extent of necrosis. This report documents that the lateral border zone of a 24-hour acute myocardial infarction consists of a histologically discrete interdigitation of peninsulas of normal and necrotic tissue with an equally discrete vascular network. Necrotic myocardium was supplied by the occluded LAD (i.e., tissue at risk), whereas normal myocardium was perfused by the non-obstructed LMCA. Except in a focal narrow zone of vascular incongruity along the lateral infarct border, no overlapping of the microcirculation was observed. We also did not find evidence of double-filled white and red vessels consistent with a microvascular collateral network connecting both the LAD and LMCA at the tissue level. Although the zone of vascular incongruity may represent a true ischemic border zone, its size suggests that at 24 hours it is inconsequential. This study thus provides an anatomical and physiological basis for our earlier observations of a histologically sharp border along the lateral boundary of an acute canine myocardial infarction. It also provides confirmation that so-called islands of myocardium, which have been considered hallucinations, may represent a significant component of the lateral infarct border.

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**Figures 3 and 4** A hematoxylin and eosin-stained section composed of an admixture of normal and necrotic tissue photographed with transillumination, and the corresponding drawing of this region based on the traced areas of necrosis, with a superimposition of the vascular supply determined by projecting the epi-illuminated photographic slide on the traced outline of the tissue. Because of cropping, the field depicted in the photograph is slightly smaller than that of the drawing.

**Figure 3** This field reveals a complex interdigitation of normal and necrotic myocardium in a highly irregular pattern. The necrotic tissue is identified by its hypereosinophilia, which was somewhat more easily appreciated when the photographic slide, from which this print was made, was projected in a darkened room upon white paper. The black dots represent Microfil within the vessels supplying both types of tissue. Only the largest and densest masses of Microfil may be seen with transillumination; with epi-illumination, many more Microfil-perfused vessels are identified, as may be appreciated by examining Figure 4. Several artifactual cracks in the tissue are present at the lateral edges of the field, and small areas of Microfil extravasation are apparent at the top (185X).

**Figure 4** The tracing of the field seen in Figure 3 shows the normal myocardium shaded and the necrotic tissue unshaded. The vessels derived from the occluded LAD (white Microfil) are shown as filled circles, and the vessels derived from the patent LMCA (red Microfil) are shown as squares. Hatched areas represent the tissue spaces, artifacts, and areas of extravasation seen in the photograph. Note that most vessels are concordant; i.e., LAD vessels supply necrotic tissue, whereas the unoccluded LMCA vessels supply normal tissue. However, it is readily apparent that some vessels are discordant (arrows). The number of such discordant vessels was determined for 10 fields from the five cases, and the mean distance between the vessels and their closest concordant border was measured. The magnitude of this distance may identify a small, focal, and non-homogeneous border region between the necrotic and normal myocardium.
marks of border zone tissue, are peninsulas supplied by conglomerous capillaries.

Investigators claiming to have identified an ischemic border zone in acute myocardial infarctions, whether based on intermediate levels of creatine phosphokinase depletion (Kjekshus and Sobel, 1970), electrocardiographic criteria (Kjekshus et al., 1972; Lie et al., 1975; Ross, 1976; Hillis et al., 1976), histochemistry (Cox et al., 1968), or ultrastructure (Buja et al., 1976; Page and Polimeni, 1977), generally have not considered the anatomical arrangement of the coronary circulation which could account for irregular zones of tissue damage with continuing viability. If one assumes that extracellular diffusion only can provide substrate to maintain the viability of extremely small volumes of tissue (possibly the size of our zone of vascular incongruity), then the survival of individual or groups of myocardial cells is clearly dependent on perfusion by contiguous capillaries. For tissue to exist in a damaged but viable state, two capillary patterns are conceivable: (1) there may be extensive interdigitation of non-connecting capillaries derived from both an occluded and a patent coronary artery, so that any one myocardial cell may be supplied with a reduced amount of substrate from the occluded vessel, and normal or increased amounts of substrate from the patent vessel or (2) capillaries may interconnect extensively, even when derived from two separate coronary arteries, so that cells are supplied with diminished but sufficient amounts of substrates to maintain viability, following occlusion of one arterial supply.

The anatomy of the canine coronary circulation down to the arterial level is well known, and there is general agreement that collateral vessels exist in the epicardial layers of the heart (Baroldi and Scomazzoni, 1965; Schaper, 1971). The arrangement of the capillary network, and particularly the existence of intercapillary anastomoses, is less well appreciated. Several investigators claimed to have identified capillary anastomoses, but their methods employed injection masses of only one color and ultrastructure (Buja et al., 1976; Page and Polimeni, 1977), generally have not considered the anatomical arrangement of the capillary network of vessels and, further, that the anatomical arrangement of the capillaries does not explain logically the existence of ischemic but viable border tissue.

General Considerations

The present study, in which tissue necrosis corresponds almost directly to the supply of that tissue by the occluded vessel, can be explained best by a discrete anatomical arrangement of the capillary circulation. The tissue at risk is defined precisely, by the methods employed herein, as the tissue supplied by the perfusion field of the occluded coronary artery. It must be stressed, however, that this study represents an evaluation of myocardial infarction at only one point in time, presumably when evolving necrosis has been completed. We have no way of evaluating the relationship of tissue necrosis and capillary circulation at earlier periods of time, because our methods require the presence of unequivocal histological evidence of cell necrosis at the light microscopic level. Studies of the early temporal progression of infarction (Cox et al., 1968; Deloche et al., 1977; Reimer et al., 1977), cannot be compared directly with our results; nor can the techniques we have employed explain the existence of damaged cells outside of the central region of necrosis (Cox et al., 1968; Deloche et al., 1977). On the other hand, ultrastructural (Buja et al., 1976) or histochemical (Cox et al., 1968) demonstrations
of presumably damaged but viable cells along the lateral border of infarcts at comparable or later times (greater than 24 hours), may require mechanisms other than the vascular supply to this tissue to explain these phenomena. In our study, we identified a 30- to 50-μm zone of seemingly viable myocardium beyond the infarct border supplied, in part, by LAD capillaries. In addition to a dual capillary supply, this region may be supplied by diffusion of sufficient substrate from the normal zone to maintain viability. Large areas of surviving myocardium supplied by either or both the LAD and LMCA were noted in the subepicardium, consistent with the presence of epicardial collaterals in the dog (Schaper, 1971).

Technical Considerations

At this point it is pertinent to discuss several of the potential limitations of this study, particularly in reference to the methodology. The design of this experiment employed simultaneous and equal pressure injections into both coronary arteries, so as to define precisely the vascular supply to the region at risk and the surrounding myocardium. Unequal injection pressures might produce perfusion of epicardial collaterals, which might result in marking of the distal vasculature with a mixture of colors. Such unequal injection pressures could be due to a variety of causes, including kinks in the catheters, obstruction of the catheter tip by the vessel wall or branch points, and thrombi or air emboli in the distal vasculature. We cannot rule out any of these possibilities with certainty; however, several general observations suggested that these potential technical problems were not significant.

In using the perfusion apparatus employed in this study, or in performing injections in large numbers of canine, rodent, and postmortem human hearts, we have observed that major obstructions to flow are rapidly apparent. These obstructions frequently require tremendous increases in perfusion pressure to overcome, or they manifest themselves by unequal flow rates of the two colors. This was not the case in the present study; rather, the capillary bed filled rapidly and, except in the necrotic zones, completely, coloring the vessels diffusely white or red. When obstruction occurred in our other studies, large areas of the myocardium did not fill with the silicone rubber, and generally only limited numbers of epicardial vessels were perfused.

Had inadequate or unequal perfusion been common, we would have expected to see double-filled vessels in the subendocardial portions of the infarct, representing collateral supply from the LMCA into the LAD. We have observed this phenomenon in postmortem human hearts injected proximal to extramural coronary artery atherosclerotic obstructions (unpublished data, 1979); however, it was not apparent in the 24-hour canine infarcts. It might be questioned whether mixing of white and red Microfil in small vessels might obscure one color, thereby producing misinterpretation of that vessel's perfusion source. This does not appear to be a significant consideration. White and red Microfil have slightly different viscosity and do not mix homogenously unless blended together mechanically. Mechanical mixing of Microfil, examined under the microscope, produced a pink color, easily discernible from the incompletely mixed colors seen in tissue sections. In zones in which we expected to see double filling of vessels because of collaterization (i.e., subepicardial myocardium), the double filling appeared either as two differently colored masses abutting one another, or as particulate contamination of one color by the other. Similar double filling of vessels by Microfil was noted in the aforementioned gracilis muscle experiments (unpublished data, 1979). We also did not observe attenuation of either color, thus suggesting that homogeneous mixing did not occur.

As noted in Results, and as illustrated in Figure 1b, rare vascular structures along the lateral border of the infarcts contained both white and red Microfil, implying collateral supply to that vessel. However in most cases we interpreted these vessels as venules, because they had a slightly larger diameter and thinner wall than the surrounding smaller capillaries or thicker-walled arterioles. We have observed a similar phenomenon in our studies of normal animals (Okun et al., 1979). In cleared tissue sections, it was possible to trace long segments of vessels from the arteriolar side of the circulation into capillaries and then into draining venules. We found that, despite the discrete nature of the arterial circulation with no collateral network connecting capillaries derived from different coronary arteries, the venous circulation occasionally drained capillaries derived from both the LAD and LMCA.

Another technical problem is also illustrated in Figure 1b. Photography of epi-illuminated sections, particularly when the vascular structures were obliquely oriented, occasionally produced red reflections on the surface of white-filled vessels. Superficially, at least, this gave the appearance of double filling; however appropriate focusing and slight tilting of the section confirmed the true nature of the perfusate.

The irregular attenuation and extravasation of the microfil in the infarct zone represent a technical limitation of this study that cannot be overcome easily. The diminution of Microfil flow to the necrotic tissue probably is a function of the "no-reflow" phenomenon described by Klener et al. (1974). These authors observed endothelial swelling and intraluminal capillary fibrin thrombi after 90 minutes of ischemia, changes which precluded perfusion by intravenous carbon black. The degree of microvascular damage after 24 hours of ischemia must be proportionately greater, and presumably
accounts for the diminished perfusion of Microfil in these zones. The extent of this phenomenon can be estimated from the data presented in Table 1. Except in rare necrotic peninsulas with completely absent filling, the reduced perfusion was not a serious limitation in evaluating the vascular supply. In fact, it provided an additional marker of tissue damage. Rapid scanning of the tissue at low power and estimation of capillary density invariably demonstrated that reduced numbers of filled capillaries were associated with tissue necrosis.

The phenomenon of Microfil extravasation in the necrotic zones also is assumed to be a result of capillary damage which allows the escape of the perfusate into tissue spaces. Support for this interpretation is provided by the absence of extravasation in the normal myocardium, suggesting that this artifact is related more to vascular injury than to the injection pressure.

Comparison with Other Studies of the "Border Zone"

Several recent studies, employing a variety of techniques, have investigated the topographical configuration of myocardial infarctions and, in particular, have investigated whether salvageable myocardium exists along the lateral border or in a transmural direction. One group (Marcus et al., 1975) used radioactive microspheres and extensive reconstruction of small segments of myocardium following circumflex coronary artery occlusion to demonstrate that a "buffer zone" of moderately ischemic myocardium does not surround the severely ischemic central zone. Another group (Barlow and Chance, 1976) used NADH fluorescence in perfused rat hearts following coronary artery ligation to show a sharp line of transition between ischemic and normal areas. More recently, the same investigators (Harken et al., 1978) employed a similar technique in canine hearts and showed that "the distance between NADH-fluorescent ischemic cells and adjacent non-fluorescent cells is less than 0.1 mm." Although these authors confirmed our previous histological findings (Factor et al., 1978), they considered that the interdigitating peninsulas of normal tissue described by us probably were supplied by coronary collateral vessels. In fact, the present report suggests that this tissue is perfused by the non-occluded coronary artery. Finally, the sharply defined histological and vascular border is consistent with our earlier report (Hirzel et al., 1977) in which we demonstrated that creatine phosphokinase (CPK) depletion is homogenous from the central to the lateral edge of the infarction zone, when tissue supplied by the non-occluded vessel is excluded from analysis.

Two current reports have presented evidence contradicting these findings and have supported the concept of a lateral border zone in acute myocardial infarction. Jugdutt et al. (1979) showed that in dogs treated with indomethacin to inhibit prostaglandin synthesis there was both lateral and subepicardial extension of acute necrosis within the region at risk. They suggested that the spatial geometry of the infarct implied the existence of a functional border zone. These investigators defined the region at risk by injecting barium sulfate-gelatin into the coronary arteries simultaneously and at the same pressure, a technique similar to ours. However, in contrast to our observations of a highly irregular but sharp border defined by Microfil and serial section histology, Jugdutt and colleagues, using serial radiographs, showed a relatively straight infarct borders. Because barium sulphate-gelatin does not penetrate the microcirculation and gross delineation of infarct extent is imprecise, this technique cannot be compared to the present histological delineation of the region of risk; these differences may account for the observed lateral extension reported in their study.

Hearse et al. (1977) concluded from metabolic, perfusion, and electrophysiological gradients in this region, that a clearly defined border zone existed. They estimated that the border zone was between 8 and 15 mm wide. However, they could not rule out the possibility that the gradients were the result of a mixed population of normal and severely damaged cells in this zone, rather than a uniform gradation of cellular injury. In a comparable study in the pig, Janse et al. (1979) concluded that, in fact, no metabolic or electrical gradients were present in the border zone after 2 hours of ischemia followed by reperfusion. These authors demonstrated that this region is composed of sharply demarcated interdigitating normal and ischemic tissue. The discrete nature of the border was similar to the histological observations made in our previous (Factor et al., 1978) and present studies, after 24 hours of ischemia.

Our investigations, showing a close correlation between the region at risk, defined by Microfil injection and the extent of tissue necrosis, are consistent with the observations of Jennings and co-workers. These authors (Lowe et al., 1978) showed that the size of an infarct in a group of dogs with proximal circumflex artery occlusion correlated closely with the size of the occluded coronary artery bed. Two other studies pertinent to the present report (Reimer et al., 1977, 1979) demonstrated that, as the duration of coronary occlusion increases, the extent of myocardial necrosis increases in a transmural direction toward the epicardium. Salvageable myocardium may exist in the subepicardium for up to 6 hours. Although our study was performed 24 hours after coronary occlusion, presumably at a time when all necrosis has taken place, the presence of histologically normal cells in the subepicardium perfused by vessels often containing red and white Microfil suggests that this tissue remains viable because of sufficient collateral supply. Another mechanism for survival of this subepicardial tissue also has been proposed by Hirzel et
al. (1976) and confirmed by Greenfield and colleagues (Bache et al., 1977), who demonstrated redistribution of coronary flow in a transmural direction toward the epicardium and away from the necrotic zone, following coronary artery occlusion.

In conclusion, this study has demonstrated that, at 24 hours after coronary occlusion, there is a close correlation between the extent of myocardial necrosis and the microvascular supply to the tissue derived from the occluded vessel. The boundaries between normal and necrotic myocardium are sharp, with no evidence of an ischemic but viable lateral border zone supplied by collateral circulation. However, we did identify a narrow focal zone of viable cells perfused by the occluded vessel, which may be supplied by diffusion from the normal region. This investigation provides an explanation for the sharpness of the lateral infarct boundary after 24 hours, and establishes conclusively that seemingly isolated fragments of tissue along the lateral border represent peninsulas supplied by the conus vessel.

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