Ultrastructural Characterization of the Border Zone Surrounding Early Experimental Myocardial Infarcts in Dogs

GEOFFREY J. GOTTLIEB, MD, SPENCER H. KUBO, MD, and DANIEL R. ALONSO, MD

From the Department of Pathology, The New York Hospital-Cornell Medical Center, New York, New York

The existence of a border zone composed of reversibly injured myocardium surrounding an evolving infarct has been the subject of controversy. In experiments designed to search for such a border zone by electron microscopy, 12 mongrel dogs underwent permanent ligation of the left anterior descending coronary artery (LAD). Two to 6.5 (average = 4.2) hours later, the hearts were excised, the area at risk (myocardium perfused by the LAD) was outlined by injection of fluorescent microspheres, and the myocardial infarct was demonstrated by the nitro blue tetrazolium (NBT) gross histochemical method. Myocardial samples for electron-microscopic study were obtained from the periphery of the infarct (tissues unstained by NBT) and serially from the immediately adjacent myocardium, which was stained deep blue by NBT. Grossly, the infarcts always involved the subendocardial myocardium, extended for a variable distance in the epicardial direction, and closely approximated the lateral margins of the area at risk. When examined by electron microscopy, the infarct periphery showed evidence of irreversible damage, thus confirming the ability of NBT to detect early myocardial necrosis. Multiple samples of the NBT-stained myocardium immediately adjacent to the infarct showed varying degrees of reversible ischemia, thus demonstrating, at the ultrastructural level, the existence of a border zone of intermediate myocardial injury. This border zone was substantial (3–4 mm in width) along the subepicardial aspect of the infarct and very thin (1–2 mm) laterally. In conclusion, a significant border zone was demonstrable by electron microscopy in the subepicardial myocardium of 8 out of 12 canine hearts with recent coronary artery occlusion. In the remaining 4 hearts, the infarcts had already reached the epicardium at the time of study, and only a thin lateral border zone was present. (Am J Pathol 1981, 103:292–303)

THE BORDER ZONE of a myocardial infarct can be conceptualized as ischemically damaged but still viable myocardium adjacent to an evolving myocardial infarct. The characterization of this zone is still incomplete, and, indeed, its very existence has become a subject of some controversy. Biochemical,[1,2] histochemical,[3–5] electrophysiologic,[1,6] and blood flow studies[1,7] have provided support for the presence of a border zone surrounding early myocardial infarcts. In contrast, other studies, using similar technics but different experimental designs, have demonstrated a sharp boundary between infarcted and normal myocardium, thus failing to show a transitional zone.[8–13] These studies have a bearing on the clinical management of myocardial infarcts, since results that question the existence of a border zone of jeopardized myocardium raise serious doubts as to whether infarct size in humans can be limited or reduced by any treatment.

The results of the studies described in this paper demonstrate the existence of a border zone ultrastructurally and determine its location within the myocardium of dogs made ischemic by permanent, experimental coronary artery occlusion.

Materials and Methods

Experimental Model

Twelve mongrel dogs of both sexes weighing 15–27 kg were anesthetized intravenously with sodium pentobarbital (30 mg/kg body weight). Each animal was

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Address reprint requests to Daniel R. Alonso, M.D., Department of Pathology, The New York Hospital-Cornell Medical Center, 525 East 68th Street, New York, NY 10021.

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intubated and placed on a Harvard respirator. The heart was exposed through a midsternal incision, the pericardium was opened, and a pericardial cradle was constructed. The left anterior descending coronary artery was ligated with a 3-0 silk at a point just distal to the first major diagonal branch, usually 2–3 cm from its origin. Following ligation, 50 mg of lidocaine was administered intravenously, and 20 mg was given every 20 minutes for 4 hours. The chest was closed, and lead II of the electrocardiogram was monitored for the remainder of the experiment. General anesthesia was maintained for the duration of the experiment. Two to 6.5 hours after coronary occlusion (average = 4.2 hours), the dogs were given 2000 units of heparin intravenously and were killed with an overdose of pentobarbital. The chest was reopened, and the heart was rapidly excised.

Demonstration of the Area Supplied by the Occluded Artery (Area at Risk)

The occlusive ligature on the coronary artery was released in 7 of the 12 hearts, and a polyethylene catheter was inserted in the artery at the precise point of occlusion. Through this catheter 10^7 fluorescent polystyrene microspheres (Duke Scientific Corp., Palo Alto, California) were injected. The microspheres measured 9–15 μ in diameter and were suspended in 5 ml of normal saline containing 0.05% Tween-80. Subsequent viewing of heart slices under ultraviolet light demonstrated fluorescence of the territory supplied by the occluded artery thus outlining the myocardium at risk of ischemic necrosis.

Visualization of Myocardial Necrosis

The hearts were then sectioned from apex to base in a plane parallel to the atrioventricular groove into serial 7–10-mm slices. The slices were incubated in a solution of nitro blue tetrazolium (NBT) in Sorensen's phosphate buffer as described by Nachlas and Shnitka.[14] Incubation was at 37 C and lasted for 7 minutes. This gross histochemical method stains the normal myocardium deep blue, whereas areas of myocardial necrosis remain unstained. Infarcts are sharply outlined by the NBT method.

During the last minute of incubation, a representative slice chosen for subsequent sectioning and study (2–4 cm from the apex) was photographed on Polaroid film while still in the NBT solution.

Electron Microscopy (Figure 1)

Two strips of contiguous 1-mm cubes were removed from the infarct periphery and immediately adjacent, noninfarcted myocardium. Up to 7 1-mm cubes were obtained from the subepicardial (8 of 12 dogs) and lateral (8 of 12 dogs) aspects of each infarct. Subepicardial sections were not taken in those instances where the infarct was transmural or nearly transmural. Control myocardial samples were taken from the midportion of the posterior left ventricular wall. The myocardial samples were then minced, fixed in 6% glutaraldehyde in 0.1 M cacodylate buffer (pH = 7.4), postfixed in 1% osmium tetroxide, dehydrated in a graded series of alcohols, and embedded in Epon. One-micron thick sections were stained with alkaline toluidine blue and examined by light microscopy. Only longitudinally oriented myofibers nearest the center of the tissue block were selected for ultrathin sectioning with an LKB Ultratome III ultramicrotome. An average of 2 tissue blocks per sampled area were processed in this fashion. The thin sections were stained with 7% uranyl acetate and lead citrate and examined with Siemens Elmiskop I and RCA EMU-3G electron microscopes. Approximately 30 to 60 myofibers were evaluated for each 1-mm region studied.

Semiquantitative Evaluation of the Infarct Periphery and Adjacent Myocardium

Ultrathin sections taken from the periphery of the infarct (area unstained by NBT) and the adjacent, NBT-stained myocardium were examined with the electron microscope and evaluated for the presence and severity of nine ultrastructural changes generally accepted as indicative of ischemic injury.[2,15–23] These changes were sarcoplasmic edema, nuclear chromatin clumping of myocytes, nuclear chromatin clumping of endothelial cells, sarcomere relaxation, sarcomere contraction, myofibrillar contraction bands, mitochondrial swelling, electron-dense mitochondrial densities, and sarcoplasmic lipid droplets. Each of these changes were graded from 0 to 4+, and the scores for all the changes were added to obtain a composite ischemic score. The ischemic score could therefore vary from 0 to 36 (9 × 4).

The range of changes in each of the nine categories was graded as follows:

1. Sarcoplasmic edema: Subsarcolemmal blebs (1+) to 50% or greater expansion of the cell volume (4+).
2. Sarcomere relaxation (stretching): The I-band with sharp border equal to ¼ the width of the A-band (1+) to the I-band equal to the width of the A-band (4+).
3. Mitochondrial swelling: Mild separation of

cristae (1+) to greater than doubling of the volume (4+).

4,5. Nuclear chromatin clumping (myofiber and endothelial): Slight granular clumping of chromatin (1+) to marked coarse clumping and margination with no finely dispersed chromatin remaining (4+).

The grading of these five changes also included consideration of cell-to-cell variation. For example, if half the cells in a studied area showed 4+ mitochondrial swelling and the other half displayed 1+ swelling, then the final grade for mitochondrial swelling would be the average of the two scores (ie, (4 + 1)/2 = 2.5).

6. Mitochondrial densities: Stacking of cristae with rare densities (1+) to large electron-dense inclusions present in most mitochondria (4+).
7. Lipid droplets: Occasional rare droplet found with some difficulty (1+) to numerous droplets present in most cells (4+).
8. Sarcomere contraction: Contracted sarcomeres with obliteration of the I-band focally present in one quarter or less of the myofibers (1+) to being present in all of the myofibers (4+).
9. Contraction bands: Markedly hypercontracted segments of myofibrils involving several sarcomeres with marked attenuation or absence of myofibrillar material on either side of this area; present rarely (1+) to being prominent in most cells (4+).

Ischemic scores were computed while the specimens were being viewed with the electron microscope and from electron photomicrographs of representative fields blindly evaluated by two observers. The ischemic scores derived by these two methods differed by no more than 3 points. Because of the essential concordance of the two scores and because a larger portion of the specimen was evaluated when the score was determined while viewing in the electron microscope, this latter score was employed.

The Student *t* test for two means was employed in the statistical analysis.

Light Microscopy

Blocks of myocardium were taken adjacent and parallel to the areas sampled for electron microscopy. The tissues were fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned at right angles to the plane of the serial gross heart slices, and stained with hematoxylin and eosin and periodic acid-Schiff stains.

Results

Gross Appearance of Myocardial Infarcts (Figure 2)

Acute myocardial infarcts were present in all 12 dogs and were clearly outlined by the NBT reaction (Figure 2). All infarcts were in an anteroseptal position and involved variable portions of the ventricular wall (Table 1). Infarcts usually occupied the inner two thirds of the wall following 4–5 hours of permanent ischemia. With one exception (Dog 191, 2.5-hour-old infarct), transmural infarcts were seen following at least 5 hours of permanent coronary occlusion.

Relationship Between Area at Risk and Myocardial Infarction (Figure 3)

The area at risk was visually determined by the presence of fluorescent microspheres in the region supplied by the occluded artery. In all cases, the lateral borders of the infarct approximated the fluorescent boundaries of the area at risk. Noninfarcted portions of the area at risk (ie, fluorescent regions stained by NBT) were only present to any appreciable extent between the epicardium and the subepicardial border of the infarct. In transmural infarcts, therefore, the infarct nearly filled the entire area at risk.

Light Microscopy

Since the sections for light microscopy were taken perpendicular to the plane of the heart slices, the resultant light-microscopic sections had the insoluble formazan pigment (from the NBT reaction) present on the edge. In this way, the areas grossly identified as

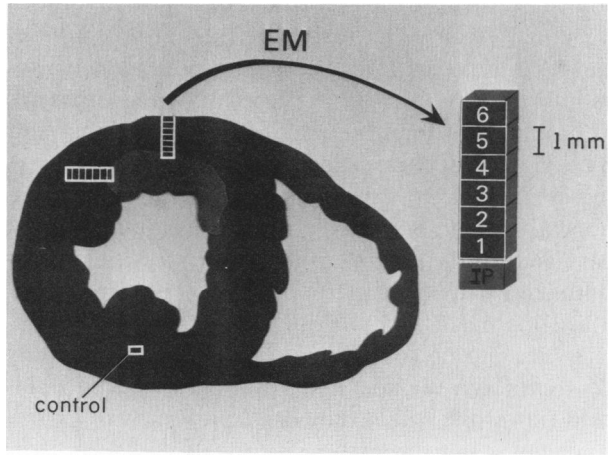


Figure 1—Serial 1-mm cubes beginning at the infarct periphery (*IP*) and extending into the surrounding noninfarcted myocardium are sampled from the lateral and subepicardial aspects of the infarct. The control is taken from the midmyocardium of the posterior wall.

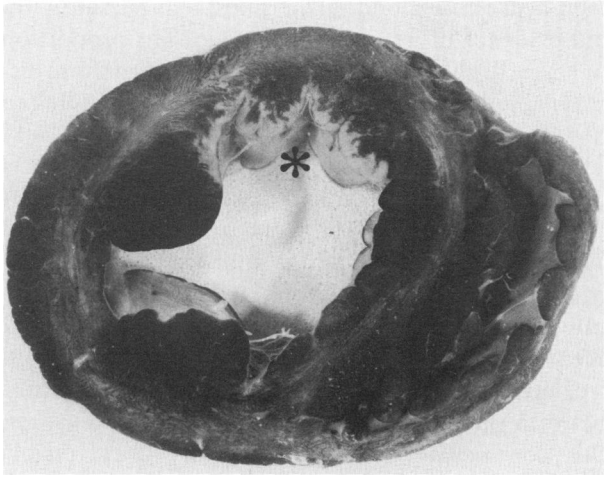


Figure 2—A slice of heart stained with Nitro blue tetrazolium. An anteroseptal infarct (*) is sharply demarcated, failing to stain with NBT.

normal and infarcted by the NBT reaction could be studied histologically. The areas devoid of formazan precipitates on the edge (infarct) showed wavy hypereosinophilic myofibers with numerous contraction bands and absence of glycogen on PAS staining. Immediately beneath the NBT-stained surface, the myocardium appeared unremarkable on hematoxylin and eosin sections, although it showed variable glycogen depletion on the lateral and subepicardial borders of the infarct. Glycogen depletion was more evident in the subepicardial border zone.

Ultrastructural Evaluation (Table 1)

Application of the composite ischemic score to the semiquantitative evaluation of evolving myocardial infarcts gave the following results: Overall, the scores ranged from 1 to 24. With one exception, sections with an ischemic score greater than 14 contained electron-dense mitochondrial densities, a finding indicative of irreversible myocardial damage.[20] Conversely, with only three exceptions, mitochondrial densities were not present in sections with ischemic scores smaller than 14. Thus, a composite ischemic score greater than 14 was generally indicative of irreversible cell damage. Myofibrillar contraction bands were present in approximately half of those sections containing electron-dense mitochondrial densities; however, they were occasionally seen in areas with ischemic scores of less than 14 and therefore without mitochondrial densities. In addition, contraction bands were also seen in a single section of control myocardium (Dog 145). No electron-dense mitochondrial densities were seen in any samples of control myocardium.

The other seven ultramicroscopic changes used in deriving the ischemic score were intermittently present in all of the areas sampled and tended to be more marked in the infarct and regions nearest the infarct.

Control Myocardium (Figure 4)

The ischemic scores of all sections obtained from the midportion of the posterior left ventricular wall ranged from 1 to 7 (average = 4.1). These values resulted from mild to moderate changes in some or all of six of the nine graded categories. Lipid droplets and electron-dense mitochondrial densities were never seen. Contraction bands were seen in only a single section (Dog 145). These changes were most likely auto-

Table 1—Ischemic Scores

			Subepicardial border zone					Lateral border zone				
Dog	Infarct age (hours)	Transmural extent	Control myocardium	Infarct periphery	1–2 mm	3–4 mm	5–6 mm	Control myocardium	Infarct periphery	1–2 mm	3–4 mm	5–6 mm
148	2	S	3.0	15.0	18.0	13.0	4.5	ND	ND	ND	ND	ND
191	2.5	T	ND*	ND*	ND*	ND*	ND*	5.0	24.0	9	7.0	5.0
145	4	S	7.0	8.0	15.5	4.5	5.5	ND	ND	ND	ND	ND
149	4	S	1.0	17.5	4.5	11.0	5.5	ND	ND	ND	ND	ND
151	4	M	1.5	16.5	8.4	7.0	†	1.5	10.5	4.25	3.75	3.0
155	4	S	2.5	17.25	13.0	13.0	2.0	ND	ND	ND	ND	ND
183	4	S	4.0	17.0	12.5	7.25	7.0	4	17.0	14.5	8.0	5.0
185	4.5	M	5.5	18.0	6.5	8.5	†	5.5	12.5	7.5	8.5	6.0
179	4.7	M	5.5	17.5	9.25	6.5	†	5.5	19.0	15.0	4.5	4.5
187	5	T	ND*	ND*	ND*	ND*	ND*	4.5	16.0	4.5	3.0	3.5
142	5.5	T	ND*	ND*	ND*	ND*	ND*	3.5	22.5	17.25	4.0	5.5
190	6.5	T	ND*	ND*	ND*	ND*	ND*	6.0	19.5	14.75	5.0	6.0
Mean	4.2		3.8	15.8	11.0	8.8	4.9	4.4	17.6	10.8	5.5	4.8
SEM			0.8	1.2	1.6	1.1	0.8	0.5	1.6	1.8	0.7	0.4

S = subendocardial; M = midmyocardial; T = transmural. ND = no samples taken.
* No epicardial samples since infarct is transmural.
† No samples at 5–6 mm since infarct is less than 5 mm from epicardium.

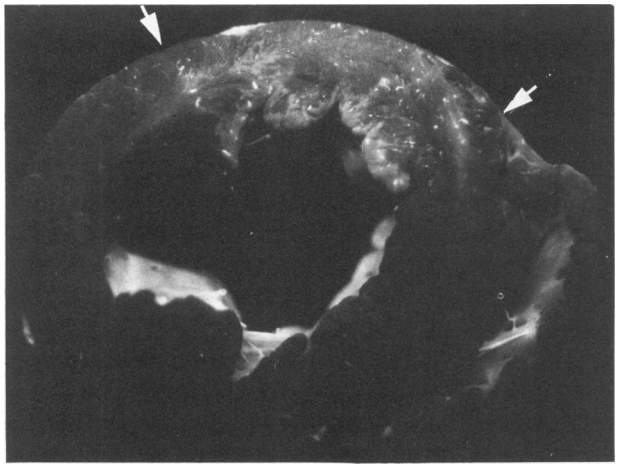


Figure 3—The same slice as seen in Figure 2, viewed under fluorescent light. The area supplied by the occluded artery (area at risk) can be seen as an area of fluorescent stippling. Note that the lateral edges of the infarct closely approach the lateral boundaries of area at risk (*arrows*).

lytic[20,22] and a consequence of the short period of warm NBT incubation (37 C) that preceded glutaraldehyde fixation.

Infarct Periphery (Figures 5 and 6)

Sections taken from the infarct edge yielded the highest ischemic scores in all but 2 of 16 samples (145, 148), and in those cases the immediately adjacent area displayed the highest score. No significant differences were noted between infarct peripheries with regard to infarct age or whether the samples were taken in either an epicardial (mean ischemic score = 15.8 ± 1.2) or lateral (mean ischemic score = 17.6 ± 1.6) direction. All but two of the sampled areas (14/16) displayed electron-dense mitochondrial densities in most or all of the cells, indicating irreversible injury. Other changes, especially clumping of nuclear chromatin, were present in variable but often marked degree in myocytes with and without mitochondrial densities. Contraction bands were present in half of the samples.

Border Zone (Figures 7, 8, and 9)

Immediately adjacent to the infarct, in the first 1–2 mm of NBT-stained myocardium, the average composite ischemic score of the samples taken from the epicardial and lateral borders were 11 ± 1.6 and 10.8 ± 1.8, respectively. No significant differences in this area were noted as a function of infarct age or whether the sample was lateral or epicardial. Ischemic changes in this area were severe but not as extensive or as homogeneous as those seen in the infarct periphery. Ischemic changes ranged from severe (6/16, with electron-dense mitochondrial densities) to moderate without evidence of irreversible damage. The proportion of severely damaged cells as compared with those displaying mild to moderate damage varied from dog to dog and was not predictable.

Sampling 3–4 mm into the NBT-stained myocardium adjacent to the infarct revealed interesting differences between the epicardial and lateral borders. The average ischemic score in samples taken in an *epicardial* orientation was 8.8 ± 1.1. This score was significantly different from both the ischemic score of the infarct periphery ($P < .0005$) and that of the control myocardium ($P < .005$). Although intermediate between the infarct and control myocardium values, this score was not due to an admixture of irreversibly injured and normal cells; rather, it was the result of variable degrees of change in categories other than mitochondrial densities and contraction bands. Most cells displayed some injury though the type and degree of change was variable from cell to cell. Irreversibly injured cells containing electron-dense mitochondrial densities were a distinct rarity. In fact, they were seen in only one instance (Dog 155). In general, the largest ischemic scores were present adjacent to infarcts with the least transmural extension.

In contrast to findings in the epicardial direction, the myocardium sampled 3–4 mm away from the *lateral* edge of the infarct failed to show any significant elevation in the ischemic score. The average ischemic score for this area was 5.5 ± 0.5, which was not significantly different from the average control value of 4.4 ± 0.5 ($P < 0.2$). The infarcts in which the lateral edges were evaluated (average = 4.6 hours), however, tended to be slightly older than those used for epicardially oriented sampling, which were all less than 4.8 hours old (average = 3.9 hours; Table 1). Even when the older transmural infarcts were excluded from consideration and only those less than 4.8 hours old (5 hearts) were studied, the average ischemic score 3–4 mm lateral to the infarct edge was still not significantly different from the average ischemic score of control myocardium ($P < 0.2$). No ultrastructural evidence of irreversible ischemic damage was seen in this area.

At 5–6 mm from the infarct edge, the average value of the ischemic score for both the epicardially (4.9 ± 0.8) and laterally (4.8 ± 0.4) oriented tissue sections was not statistically different from the average control values (3.8 ± 0.8 and 4.4 ± 0.4, respectively). The types and magnitude of the ultramicroscopic changes were similar to those seen in the controls.

The average ischemic scores for all the sampled areas are graphically shown in Figures 10 and 11.

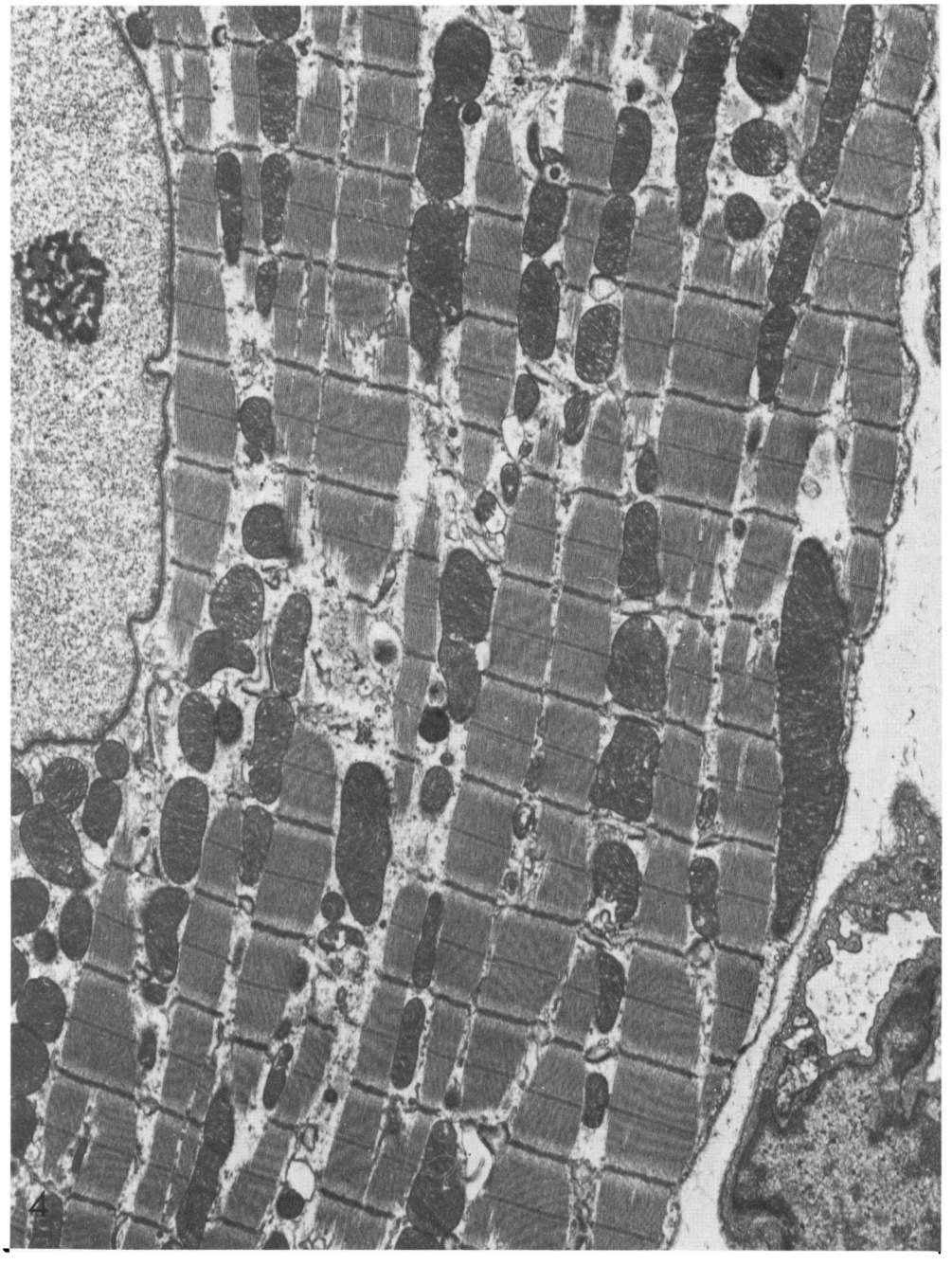
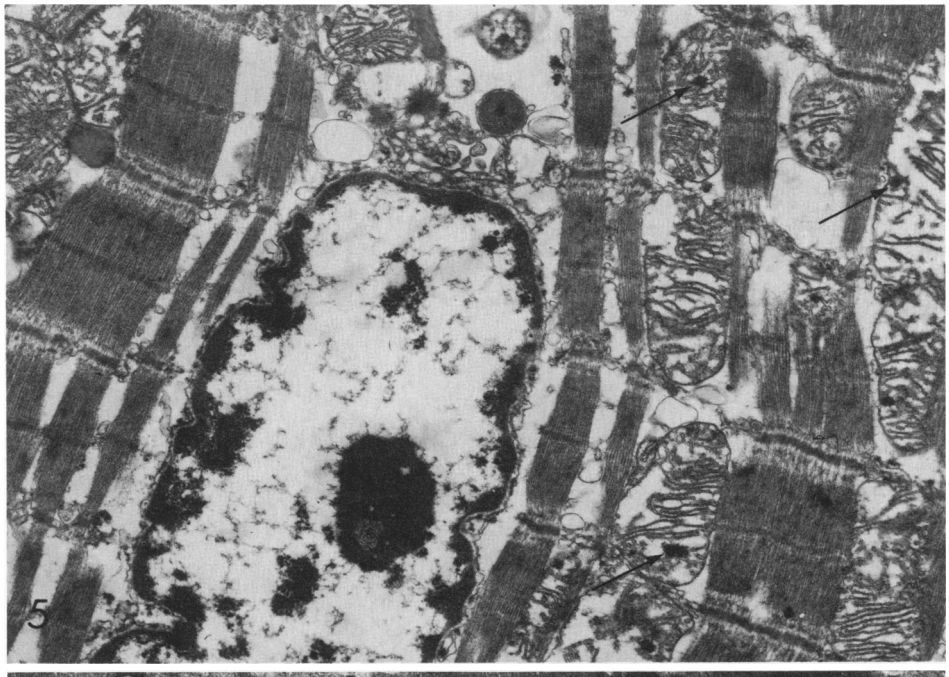


Figure 4—Control myocardium. The nucleus and mitochondria are normal. Mild changes include slight sarcomere relaxation with clearly visible I-bands and a small amount of sarcoplasmic expansion. A normal capillary is also present. (×7800)

Ultrastructural Correlation Between Infarcted and Border-Zone Myocardium and the NBT Histochemical Reaction

Ultrastructural evaluation of the infarct and surrounding myocardium, as outlined by the NBT reaction, confirmed that this method accurately identified necrotic myocardium. NBT-negative areas showed the most severe ischemic changes and had high ischemic scores characteristic of infarcted myocardium. NBT-positive areas, however, showed a gradation of ischemic change ranging from moderate to severe in the myocardium immediately adjacent to the infarct to changes comparable to those of control myocardium a few millimeters away from the edge of the infarct. Thus, the border zone myocardium was stained



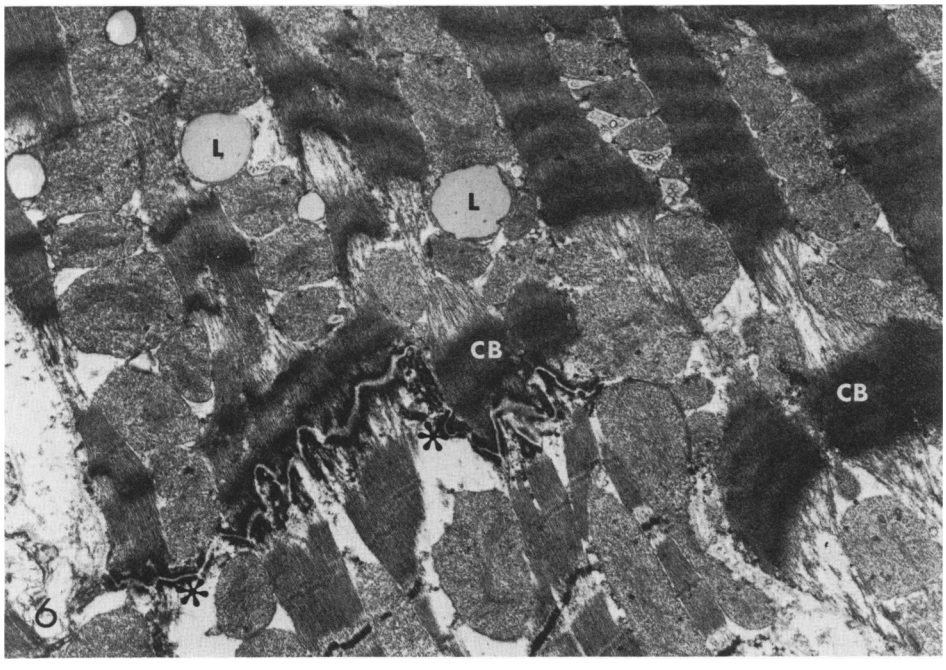


Figure 5—Irreversibly injured myocardium from the infarct periphery. There is marked clumping and margination of nuclear chromatin. The mitochondria are significantly swollen and contain numerous densities (*arrows*). In addition, there is moderate sarcomere relaxation with obvious I-bands. Sarcoplasmic expansion is also present. (× 10,200)
Figure 6—Irreversibly injured myocardium from the infarct periphery. Most prominent are the contraction bands (*CB*). The adjacent cell inferior to the intercalated disc (*) contains relaxed sarcomeres. In spite of the lack of significant mitochondrial swelling, densities are numerous. Lipid droplets (*L*) are also focally numerous. (× 10,200)

by NBT in spite of the fact that it showed significant ultrastructural changes indicative of ischemic injury.

Discussion

The results of this study indicate that evolving myocardial infarcts in the dog are surrounded by a zone of ischemically damaged but still viable myocardium. Cells in this border zone appear normal in histologic sections stained with hematoxylin and eosin, are glycogen-depleted, and show mild to moderate ischemic changes when examined electron-microscopically. The border zone is immediately adjacent to the periphery of myocardial infarcts resulting from 2 to 6.5 hours (average = 4.2 hours) of permanent coronary occlusion. It measures up to 4 mm in width at the subepicardial edge of the infarct and tends to be narrower (1–2 mm) along the lateral aspect, where the edge of the infarct closely approaches the lateral boundaries of the territory supplied by the occluded artery, ie, the area at risk (Figure 12).

The electron-microscopic changes of myocardial is-

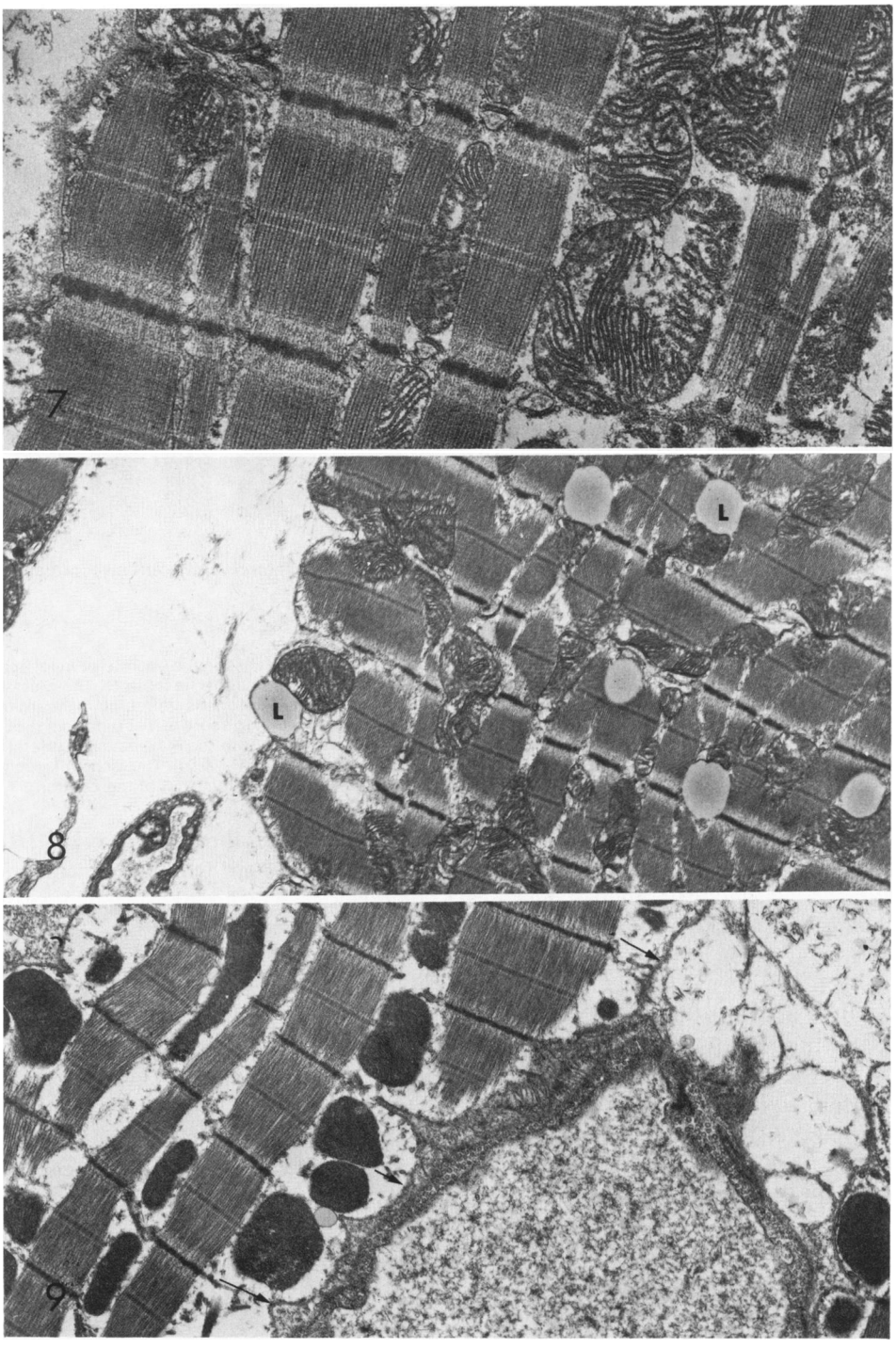


Figure 7—Myocardial sample taken 3–4 mm from the infarct edge (border zone). There is moderate sarcomere relaxation and mitochondrial swelling. (×13,400) **Figure 8**—Myocardial sample taken 3–4 mm from the infarct edge (border zone). In addition to slight sarcomere relaxation and minimal mitochondrial swelling, there are numerous lipid droplets (*L*) in the sarcoplasm. (×6800) **Figure 9**—Myocardial sample taken 3–4 mm from the infarct edge (border zone). Prominent sarcoplasmic edema is evident with lifting of the sarcolemmal membrane (*arrows*). The mitochondria and sarcomeres are normal. (×7700)

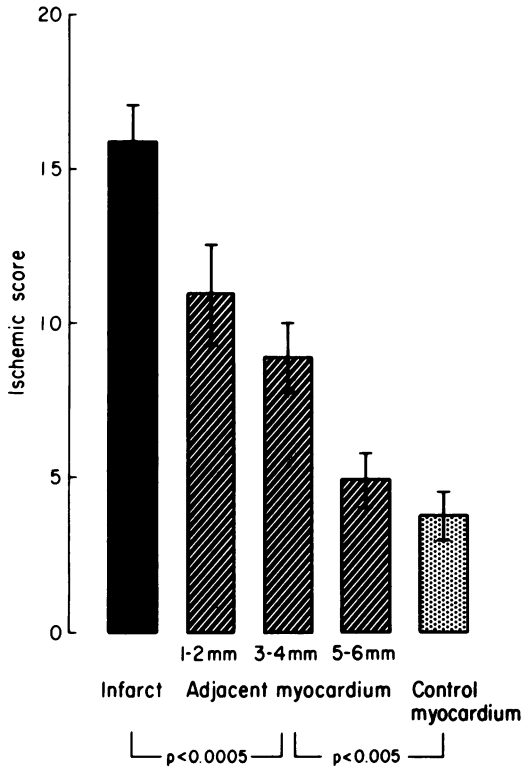


Figure 10—Average ischemic scores for myocardial samples taken from the *subepicardial* aspect of the infarcts. The cross-hatched bars indicate samples taken from the noninfarcted myocardium adjacent to the infarcts. The ischemic score at 3–4 mm from the infarct edge is significantly different from the scores of both the infarct periphery ($P < 0.0005$) and control myocardium ($P < 0.005$).

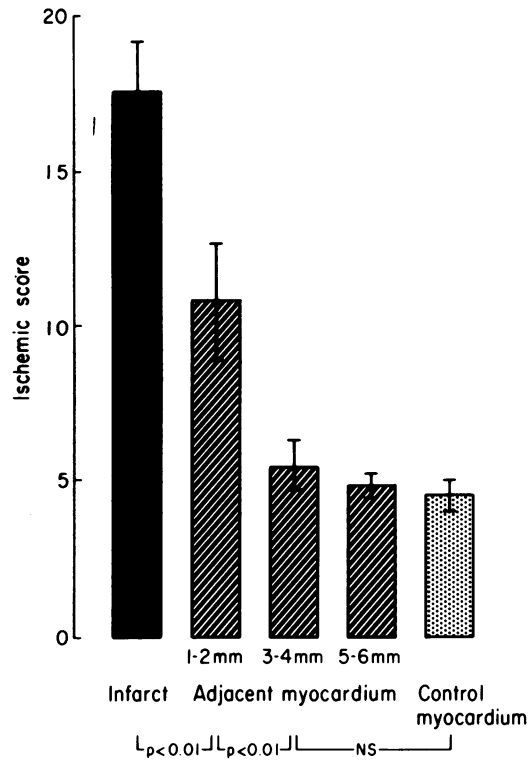


Figure 11—Average ischemic scores for myocardial samples taken from the *lateral* aspect of the infarcts. The cross-hatched bars indicate samples taken from noninfarcted myocardium adjacent to the infarcts. The ischemic score at 1–2 mm from the infarct edge is significantly different from those of both the infarct ($P < 0.01$) and the control myocardium ($P < 0.01$). The ischemic score at 3–4 mm is not statistically different from that of the control.

chemia and necrosis have been well described.[2,15–23] It is generally accepted that the appearance of electron-dense deposits in the mitochondrial matrix indicates irreversible cell damage.[20] Observations reported here are in harmony with this concept. Thus, ultrastructural evidence of cell death as indicated by the presence of mitochondrial densities was seen only in myocardial samples having large ischemic scores which were obtained from the periphery of or immediately adjacent to the infarcted tissue.

Although we computed the ischemic scores, giving equal weight (0–4+) to all nine studied ultrastructural changes and without regard to the magnitude of collateral blood flow present in each of the sampled areas, the scores displayed a definite progression from normal to infarcted tissue, and there was a clear cutoff point (ischemic score = 14) between areas with lethal and reversible ischemic damage. To have singled out criteria indicative of lethal injury (ie, mitochondrial densities) at the inception of the study and arbitrarily given them more weight in the derivation of the ischemic score would have been inappropriate, since it would have biased the ischemic scores of lethally damaged tissue toward higher values. Furthermore, tissue for ultrastructural study was taken from the edge of the infarct and not from the central and subendocardial regions, which are known to have the lowest levels of collateral blood flow.[7]

The existence of a border zone has been the subject of controversy. Data from histochemical,[3,5] biochemical,[1,2] regional blood flow,[1,7] and electrophysiologic[1,6] experiments have provided evidence for the existence of such a border zone. In addition, there is a large body of evidence suggesting that the eventual size of an infarct can be reduced by various therapeutic interventions.[24–31] The decreased size of the infarct in these instances might well have been due to the salvaging of portions of the border zone. Functionally, therefore, the border zone can be conceptualized as being composed of myocardial tissue that remains viable following treatment, despite reductions of blood flow which result in necrosis in untreated animals.[28,32,33]

Implicit in the argument of those who say that a border zone does not exist[8–13] is the concept that myofibers die in an "all or none" fashion and that ischemically altered but viable cells are present in insignificant numbers or not at all. In favor of the existence of

a border zone is the accumulated evidence on the pathogenesis of cell injury.[34,35] Cells exposed to injurious conditions (eg, ischemia) undergo biochemical and morphologic changes, eventually pass beyond an as yet poorly understood threshold, and die. Before this threshold is reached, however, the changes are potentially reversible if the harmful conditions are removed. Alternatively, the cell may achieve a new level of steady state if the harmful agents remain present in sublethal amounts. Our findings support this concept, since the border zone is characterized by myocytes displaying various degrees of sublethal injury rather than a mixture of viable and dead cells.

Several experimental studies have been interpreted as providing evidence that negates the existence of the border zone. Histologic[12] and enzymatic[11] (CPK) evaluation of canine infarcts point out that the boundary of an infarct is irregular, with interdigitation of viable and dead myocardium. Samples obtained from this area reflect a mixture of dead and normal cells and thus explain the intermediate values reported by other investigators as proof of the existence of a border zone. The infarcts in the above studies, however, were 24 hours old, and it is very likely that by then no border zone remains, since the infarct is completed. Indeed, by 6 hours most of the salvageable myocardium is dead.[33] In the present study we noted the irregularity of the infarct border and were careful to choose areas for electron microscopy that were clearly noninfarcted by NBT staining.

Marcus and associates used a study of regional myocardial blood flow to refute the concept of the border zone.[8] In this experiment, large myocardial samples were studied and measurements made after five minutes of coronary artery occlusion. Since irreversible ischemic damage to myocardial cells does not occur until at least 20 minutes after occlusion,[19,20,36] it could be argued that, 5 minutes after occlusion, the bulk of the ischemic area is reversibly damaged and amenable to salvage by an intervention such as reflow, thus fulfilling the functional definition of the border zone.[28,32,33] In other words, after only 5 minutes of occlusion all ischemic tissue is part of the border zone, since at that time it is only sublethally damaged. A similar argument can be used to criticize the interpretation of studies employing NADH fluorescence as a measurement of irreversible ischemia[9,10] 5 minutes after coronary occlusion.

In a recent study[13] Janse and associates, using electrophysiologic, biochemical, and histochemical parameters, concluded that no demonstrable border zone could be identified in the pig following 2 hours of high LAD ligation. They pointed out, however, that very early interventions might well have prolonged cell survival in the region surrounding the infarct. They also suggested that the characteristics of a border zone are at least in part determined by the presence of collateral circulation, which is significantly less developed in the pig than in dogs or humans.

Although not the original objective of this study, observations reported here constitute an ultrastructural verification of the validity of the NBT reaction. Thus, areas of myocardium that failed to stain with NBT showed irreversibly damaged cells when examined with the electron microscope. However, the border zone, composed of myocardial cells showing predominantly mild to moderate ischemic changes and absence of electron-dense deposits in mitochondria, was stained by NBT, indicating preservation of dehydrogenase activity.[14]

Based on the results of the present study, we conceptualize the border zone as an area of reversibly damaged myocardium adjacent to an evolving infarct and within the area supplied by the occluded artery (area at risk). It can be shown by electron microscopy to extend up to 4 mm beyond the subepicardial edge of infarcts less than 5 hours old. Laterally this zone is thinner (less than 2 mm); and, in fact, the infarct closely approaches the lateral boundaries of the area at risk (Figure 12). Older infarcts generally display greater transmural extension. It appears, therefore, that an infarct extends to the lateral borders of the area at risk and then spreads by transmural extension through the ischemic border zone as a function of time. This concept of infarct progression has been previously demonstrated and termed the "wavefront of myocardial necrosis" by Reimer et al.[32,33] As the in-

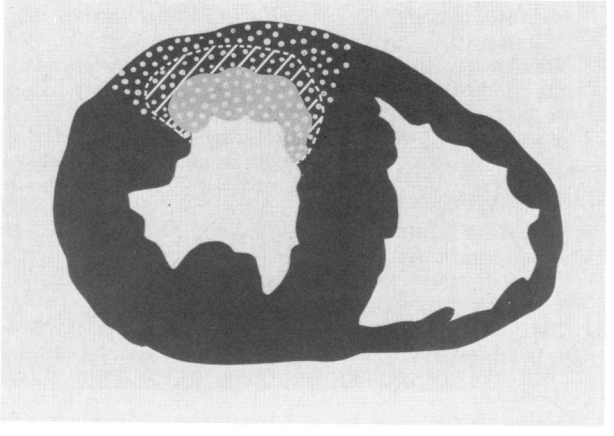


Figure 12—The acute infarct (*gray*) of approximately 4-hour duration is subendocardial in location and closely approaches the lateral edges of the area at risk (*stippled area*). Ischemically damaged but largely viable myocardium (the ischemic border zone) is adjacent to the infarct (*cross-hatched area*). This border zone is widest along the subepicardial aspect of the infarct (3–4 mm) and is narrower laterally (1–2 mm).

farct progresses, a variable amount of border zone myocardium predominantly in a subepicardial location is kept viable by preexisting collateral circulation and diminished metabolic demands (ie, cessation of contraction) for up to 6 hours.[32,33]

It is hoped that the time necessary for an infarct to become transmural is longer in man than it is in the canine model. Therapies designed to preserve and protect ischemic myocardium would then salvage the border zone myocardium, thus preventing the development of transmural infarction and its complications: pump failure, ventricular wall rupture, and ventricular aneurysm.

References

1. Hearse DJ, Opie LH, Katzeff IE, Lubbe WF, Van Der Werff TJ, Peisach M, Boulle G: Characterization of the "border zone" in acute regional ischemia in the dog. Am J Cardiol 1977, 40:716-726
2. Bilheimer DW, Buja LM, Parkey RW, Bonte FJ, Willerson JT: Fatty acid accumulation and abnormal lipid deposition in peripheral and border zones of experimental myocardial infarcts. J Nucl Med 1978, 19: 276-283
3. Cox JL, McLaughlin VW, Flowers NC, Horan LG: The ischemic zone surrounding acute myocardial infarction: Its morphology as detected by dehydrogenase staining. Am Heart J 1968, 76:650-659
4. Dusek J: Significance of morphological changes at the periphery of an experimental myocardial infarct. Rec Adv Stud Cardiac Struct Metab 1972, 1:430-438
5. Fishbein MC, Hare C, Gissen S, Maclean D, Maroko PR: Histochemical identification and quantification of border zones during the evolution of myocardial infarction in the rat (Abstr). Circulation 1977, 55, 56(Suppl III):III-71
6. Cox JL, Daniel TM, Boineau JP: The electrophysiologic time-course of acute myocardial ischemia and the effects of early coronary artery reperfusion. Circulation 1973, 48:971-983
7. Vokonas PS, Malsky PM, Paul SJ, Robbins SL, Hood WB: Radioautographic studies in experimental myocardial infarction: Profiles of ischemic blood flow and quantification of infarct size in relation to magnitude of ischemic zone. Am J Cardiol 1978, 42:67-75
8. Marcus ML, Kerber RE, Ehrhardt J, Abbound FM: Three dimensional geometry of acutely ischemic myocardium. Circulation 1975, 52:254-263
9. Barlow CH, Chance B: Ischemic areas in perfused rat hearts: Measurement by NADH fluorescence photography. Science 1976, 193:909-910
10. Harden W, Barlow C, Harken A: High resolution, three dimensional display of myocardial ischemic "Borderzone" in dogs. Circulation 1978, 57, 58(Suppl II):II-35
11. Hirzel HO, Sonnenblick EH, Kirk ES: Absence of a lateral border zone of intermediate creatine phosphokinase depletion surrounding a central infarct 24 hours after acute coronary occlusion in the dog. Circ Res 1977, 41: 673-683
12. Factor SM, Sonnenblick EH, Kirk ES: The histologic border zone of acute myocardial infarction—islands or peninsulas? Am J Pathol 1978, 92:111-124
13. Janse MJ, Cinca J, Morena H, Fiolet JWT, Kléber AG, De Vries GP, Becker AE, Durrer D: The "Border Zone" in myocardial ischemia: An electrophysiologic, metabolic and histochemical correlation in the pig heart. Circ Res 1979, 44:576-588
14. Nachlas MM, Shnitka TK: Macroscopic identification of early myocardial infarcts by alterations in dehydrogenase activity. Am J Pathol 1963, 42:379-405
15. Caulfield J, Klionsky B: Myocardial ischemia and early infarction: An electron microscopic study. Am J Pathol 1959, 35:489-524
16. Korb G, Totovic V: Electron microscopical studies on experimental ischemic lesions of the heart. Ann NY Acad Sci 1969, 156:48-60
17. Jennings RB, Baum JH, Herdson PB: Fine structural changes in myocardial ischemic injury. Arch Pathol 1965, 79:135-143
18. Jennings RB, Sommers HM, Herdson PB, Kaltenback JP: Ischemic injury of myocardium. Ann NY Acad Sci 1969, 156:61-78
19. Kloner RA, Ganote CE, Whalen DA, Jennings RB: Effect of a transient period of ischemia on myocardial cells: II. Fine structure during the first few minutes of reflow. Am J Pathol 1974, 74:399-422
20. Jennings RB, Ganote CE: Structural changes in myocardium during acute ischemia. Circ Res 1974, 34, 35(Suppl III):156-168
21. Trump BF, Mergner WJ, Kahng MW, Saladino AJ: Studies on the subcellular patholphysiology of ischemia. Circulation 1976, 53(Suppl I):17-26
22. Armiger LC, Seelye RN, Carnell VM, Smith CU, Gavin JB, Herdson PB: Morphologic and biochemical changes in autolyzing dog heart muscle. Lab Invest 1976, 34: 357-362
23. Armiger LC, Gavin JB: Changes in the microvasculature of ischemic and infarcted myocardium. Lab Invest 1975, 33:51-56
24. Maroko PR, Braunwald E: Modification of myocardial infarction size after coronary occlusion. Ann Intern Med 1973, 79:720-733
25. Maroko PR, Braunwald E: Effects of metabolic and pharmacologic interventions on myocardial infarct size following coronary occlusion. Circulation 1976, 53 (Suppl I):162-168
26. Smith HJ, Singh BN, Nisbet HD, Norris RM: Effects of verapamil on infarct size following experimental coronary occlusion. Cardiovasc Res 1975, 9:569-578
27. Epstein SE, Borer JS, Kent KM, Redwood DR, Goldstein RE, Levitt B: Protection of ischemic myocardium by nitroglycerin: Experimental and clinical results. Circulation 1976, 53(Suppl I):191-198
28. Kloner RA, Braunwald E, Maroko PR: Long-term preservation of ischemic myocardium in the dog by hyaluronidase. Circulation 1978, 58:220-226
29. Rasmussen MM, Reimer KA, Kloner RA, Jennings RB: Infarct size reduction by propranolol before and after coronary ligation in dogs. Circulation 1977, 56:794-798
30. Maroko PR, Bernstein EF, Libby P, Delaria GA, Covell JW, Ross J, Braunwald E: Effects of intraaortic balloon counterpulsation on the severity of myocardial ischemic injury following acute coronary occlusion. Circulation 1972, 45:1150-1159
31. Mathur VS, Guinn GA, Burris WH: Maximal revascularization (reperfusion) in intact conscious dogs after 2 to 5 hours of coronary occlusion. Am J Cardiol 1975, 36:252-261
32. Reimer KA, Lowe JE, Rasmussen MM, Jennings RB: The wavefront phenomenon of ischemic cell death: I. Myocardial infarct size vs duration of coronary occlusion in dogs. Circulation 1977, 56:786-794
33. Reimer KA, Jennings RB: The "wavefront phenomenon" of myocardial ischemic cell death: II. Transmural

progression of necrosis within the framework of ischemic bed size (myocardium at risk) and collateral flow. Lab Invest 1979, 40:633–644
34. Majno G, LaGattuta M, Thompson TE: Cellular death and necrosis: Chemical, physical and morphologic changes in rat liver. Virchow's Arch [Pathol Anat] 1960, 333:421–465
35. Trump BF, Arstila A: Cellular reaction to injury, Principles of Pathobiology. Edited by M LaVia, R Hill. New York, Oxford University Press, 1975, pp 9–96
36. Jennings RB, Sommers HM, Smyth GA, Flack HA, Linn H: Myocardial necrosis induced by temporary occlusion of a coronary artery in the dog. Arch Pathol 1960, 70:68–78