Experimental Myocardial Infarction in the Rat

Qualitative and Quantitative Changes During Pathologic Evolution

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Surgical occlusion of the left coronary artery of the rat is a relatively simple, economical technique for producing experimental myocardial infarction (MI). Histologic study of 1- to 21-day-old MI in rats showed that following a mild and brief acute inflammatory response at the margins of the necrotic myocardium, there is chronic inflammation, vascular and collagenous proliferation, and resorption of necrostic tissue which progresses until scar formation is complete, usually by 21 days. From Day 1 to Day 21 the volume of infarcted myocardium decreases from 45.9 ± 5.9% (mean ± SEM) to 26.1 ± 3.2% of the left ventricle and infarct thickness decreases from 1.30 ± 0.06 mm to 0.47 ± 0.02 mm. Concomitantly, the percent of the surface area of the left ventricle which is infarcted decreases insignificantly from 55.7 ± 7.2% to 48.3 ± 4.2%, indicating that the decrease in volume of the infarcted tissue occurs primarily as a result of thinning of the MI. This study provides qualitative and quantitative information on the natural history of MI in rats, which should be useful as a baseline for future studies. (Am J Pathol 90:57–70, 1978)

EXPERIMENTAL MODELS OF MYOCARDIAL INFARCTION have provided much information concerning the morphology, biochemistry, electrophysiology, and mechanical properties of the infarcted myocardium and the hemodynamic changes that occur following coronary artery occlusion. In recent years there also has been increased interest in the identification of interventions that might reduce infarct size following coronary artery occlusion.[1–10] Experimental models used for such studies require animals in which infarcts can be produced and quantitated easily, consistently, and economically. In the dog, the animal used most frequently, coronary artery ligation requires relatively large laboratory facilities and laborious surgical procedures. The infarcts that result from ligations at similar sites of the same arteries vary greatly in size so that large numbers of these relatively expensive animals are required to achieve statistically significant results.[11,12] Techniques already available for coronary artery occlusion in small animals, such as the rat,[11,13,14] offer the following advantages: a) surgical occlusion is easy to perform and does not require

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Supported by Grant HL06370-16 from the National Institutes of Health and Contract 1-HV-53000 from the National Heart, Lung and Blood Institute. Dr. Maclean is in receipt of a British Heart Foundation-American Heart Association Research Fellowship (75-310).

Accepted for publication September 7, 1977.

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elaborate equipment or large laboratories; b) rats are more economical to purchase and maintain; and c) because the hearts of rats are small the entire heart can be sampled extensively in relatively few histologic sections and quantitation of infarcts is accomplished more easily. The rat model of myocardial infarction has been used to study morphologic features of evolving infarcts [13-16] and to assess the effect of various interventions on infarct size.[17-21] Since this model shows great promise for the study of interventions that might alter the evolution of infarction, the present study was undertaken to examine in more detail the pathologic evolution of myocardial infarction in the rat following coronary artery occlusion and to quantitate the changes that occur.

Materials and Methods

Coronary artery occlusion was performed in 80 male albino Sprague-Dawley rats weighing 250 to 300 g using techniques described in detail elsewhere.[11,13,14] Briefly, in ether-anesthetized rats 2-cm incisions to the left of and parallel to the sterni were made; the fifth and sixth ribs were separated with a clamp; and the hearts were exteriorized by applying pressure to the lateral aspects of the thoracic cage. The left coronary arteries were then occluded 1 to 2 mm from their origins with single sutures. The chests were closed and the rats allowed to recover.

One to 21 days following the occlusion, groups of 3 to 7 rats were reanesthetized and killed by excision of the heart (Table 1). The hearts were fixed in 10% phosphate-buffered formalin for 24 hours. The ventricles were sectioned from apex to base in a plane parallel to the atrioventricular groove (Figure 1). Four slices measuring 2 to 2.5 mm in thickness were obtained from each heart. Following dehydration and clearing, all four of the tissue slices from each heart were embedded. Five-micron-thick sections were cut and mounted on 2 in × 2 in glass slides and stained with hematoxylin-eosin, Masson's trichrome method to demonstrate collagen, Mallory's stain for iron, von Kossa's and Dahl's stains for calcium salts, and Fite's method for lipofuscin pigment.[22] The following features were sought specifically (Table 1): coagulation necrosis, waviness, and thinning of myocardial fibers; contraction bands; intercellular edema and hemorrhage; infiltrates of neutrophilic polymorphonuclear (PMN), lymphocytic, plasmacytic, pigmented mononuclear, and eosinophilic cells; and vascular, fibroblastic, and collagenous proliferation. All features were graded as follows: 0 = absent, + 1 = mild, +2 = moderate, and +3 = severe. These broad categories indicate the relative magnitude of histopathologic findings in infarcts at any given time and aid in assessing whether these changes increased or decreased in prominence as the infarcts evolved.

To assess changes in infarct size as the infarcts evolved, histologic sections of all four slices of each heart were projected onto a screen at a magnification of 10× and a planimeter was used[23] to make the following measurements: a) areas of infarcted and noninfarcted left ventricular myocardium and b) lengths of the portions of the circumference of the left ventricle overlying infarcted and noninfarcted myocardium. From these measurements the following were calculated for each heart: a) percent, by area, of the left ventricle which was infarcted and b) percent, by circumference, of left ventricle which was infarcted. These percentages represent estimates of the percent of the volume of the left ventricular myocardium which was infarcted and the percent of the surface area of the left ventricular wall which was infarcted. The mathematical justification for using two-dimensional tissue sections to make quantitative estimates of three-dimensional structures (stereology) has been described in detail elsewhere.[24] In addition to the planimetric measure-

Table 1—Relation of Age of Infarct to Histologic Changes

Days after occlusion	No. of animals	Necrotic changes of myocardial fibers	Wavy fibers	Neutrophils	Lympho-cytes & plasma cells	Pigmented mononuclear cells	Vascular changes		Fibroblasts	Collagen
							Congestion, edema, hemorrhage	Prolifera-tion		
1	5	+3	+3	+1	0	0	+3	0	0	0
2	5	+3	+3	+2	+1	0	+3	+1	0	0
3	6	+3	+2	+2	+1	0	+2	+2	+1	+1
4	3	+2	+1	+2	+1	0	+1	+3	+2	+1
5	4	+2	+1	+2	+2	+1	+1	+3	+3	+2
6	6	+2	+1	+2	+2	+2	+1	+3	+3	+2
7	6	+1	+1	+1	+3	+3	+1	+3	+3	+3
8	7	+1	+1	+1	+2	+3	+1	+3	+3	+3
9	5	+1	0	+1	+2	+3	+1	+3	+3	+3
11	5	+1	0	+1	+2	+3	+1	+3	+3	+3
13	5	+1	0	+1	+2	+3	+1	+3	+3	+3
15	4	+1	0	+1	+1	+3	+1	+3	+3	+3
17	6	+1	0	0	+1	+3	+1	+2	+3	+3
19	6	+1	0	0	+1	+2	+1	+2	+3	+3
21	7	0*	0	0	+1	+2	+1	+2	+2	+3

All changes graded for severity as follows: 0 = absent; +1 = mild; +2 = moderate; +3 = severe.
* In one of seven hearts a few necrotic fibers were present within the connective tissue scar.

ments described above, the thickness of the infarct was measured at the point of maximum thinning of the infarcted left ventricle wall.

Results

Coronary artery ligation in the rat resulted in a uniform sequence of histologic changes (Table 1). At each time interval following arterial occlusion there was little variation from one animal to another.

Necrotic Changes of Myocardial Fibers

At 24 hours a zone of necrotic muscle fibers was evident. At the margins of the infarct some of the fibers were thinned and wavy and were separated by spaces presumably representing interstitial edema (Figure 2). Some fibers at the edge of the infarcts contained contraction bands (Figure 3). Variable bands of myocardium immediately adjacent to the endocardium and epicardium, usually two to three fibers thick, were not necrotic but remained normal. From Day 4 to Day 21 there was gradual shrinkage of the necrotic zone. No cellular infiltrates or vascular proliferation appeared in the interior of the necrotic zone in which resorption was occurring. The necrotic cells were removed completely by Day 21 in six of seven infarcts.

Infiltration by Inflammatory Cells

At 24 hours a few PMNs were present at the margin of the necrotic area. This sparse infiltrate increased slightly in density by Day 2. From Day 2 to Day 6 PMNs were still present but then began decreasing in number. At 48 hours an occasional lymphocyte was noted at the periphery of the infarct, and by 72 hours lymphocytes and plasma cells encircled the central area of necrotic fibers. By Day 7 this infiltration of chronic inflammatory cells had peaked and then decreased gradually until Day 21 when only occasional lymphocytes and plasma cells were still present. Pigmented mononuclear cells, most of which contained hemosiderin rather than lipofucsin, appeared by Day 5 and persisted in the scar which eventually formed. Eosinophils were not observed at any time in these experimental infarcts.

Vascular Changes

Edema, vascular congestion, and focal areas of hemorrhage were evident at the edge of infarcts at 24 hours and small blood vessel proliferation was apparent at 48 hours. Such vessels had blood-filled lumens surrounded by plump endothelial cells with swollen vesicular nuclei. Occasional mitoses were noted in endothelial cells. By Day 3 the edema and

congestion had decreased. Areas of hemorrhage were present until Day 7 but were small. Vascular proliferation became quite prominent by Day 3 and remained so until Day 17, when decreased numbers of vessels were noted. However, at Day 21 dilated vessels persisted in the healed scar.

Fibroblasts and Collagen

As early as Day 3, spindle-shaped fibroblastic cells were present at the periphery of the infarct; trichrome staining showed the formation of thin collagen fibers. Some fibroblasts had plump vesicular nuclei, and mitoses were evident. From Day 4 until Day 21 there was continued prominence of fibroblasts with increased deposition of collagen until the entire necrotic zone was replaced. At the junction of normal myocardium and proliferating collagen some cells had "caterpillar" or "owl-eye" (in cross section) nuclei as described by Antischkow [25] (Figure 4). Fibroblastic and collagenous proliferation also occurred in the epicardium and endocardium adjacent to the infarcted myocardium.

Other Observations

In all rats there was consistent sparing of a thin subepicardial and subendocardial band of myocardial fibers which persisted until Day 21, although endocardial and epicardial fibrosis occurred adjacent to the zone of infarcted myocardium. Usually, the papillary muscles were also preserved in spite of infarction of the entire left ventricular free wall. Single, isolated, but morphologically normal fibers surrounded by collagen and fibroblasts were also present within the healed myocardial scar, usually around large veins. Amorphous basophilic granules, which were shown by special staining to be calcium salts, were present in fibers by Day 1 usually near the margin of the necrotic area. Occasionally, a giant-cell reaction was present around fibers which displayed marked calcification (Figure 5).

Quantitative Aspects of the Evolution of Myocardial Infarcts

In sham-operated rats there is no myocardial necrosis or fibrosis except for a small area immediately beneath the epicardium where the suture has been placed around the coronary artery, and there is no asymmetry in the thickness of the ventricular wall (left ventricular free wall to septal ratio = 1.01 ± 0.01) (mean $\pm$ SEM).[19] Planimetry of the four sections of rat hearts with infarcts (Table 2 and Figure 6) showed that from Day 1 to Day 21 there was progressive thinning of the infarcted wall from 1.30 ± 0.06 mm ($N = 5$ rats) to 0.47 ± 0.02 mm ($N = 7$ rats) ($P < 0.001$). At the same time, the volume of the left ventricle involved decreased from $45.9 \pm 5.9\%$ (N

Table 2—Quantitative Aspects of the Evolution of Myocardial Infarction

Days after occlusion	No. of animals	Thickness of infarcted LV wall (mm)*	Infarct volume (% of LV)	Infarct surface area (% of LV)
1	5	1.30 ± 0.06†	45.9 ± 5.9†	55.1 ± 7.2†
2	5	1.20 ± 0.14	40.6 ± 7.0	56.1 ± 6.3
3	6	0.88 ± 0.07	39.8 ± 4.1	57.9 ± 3.2
4	3	0.83 ± 0.18	39.9 ± 5.4	56.3 ± 7.1
5	4	0.75 ± 0.05	36.6 ± 3.1	59.2 ± 4.8
6	6	0.75 ± 0.03	28.8 ± 4.2	50.2 ± 5.2
7	6	0.75 ± 0.07	25.5 ± 4.1	51.1 ± 4.7
8	7	0.71 ± 0.05	24.2 ± 2.1	49.8 ± 3.9
9	5	0.56 ± 0.04	29.6 ± 2.4	50.3 ± 5.4
11	5	0.64 ± 0.05	26.8 ± 5.7	47.1 ± 5.0
13	5	0.58 ± 0.04	22.8 ± 2.9	43.7 ± 0.9
15	4	0.55 ± 0.06	23.0 ± 3.2	46.3 ± 2.8
17	6	0.48 ± 0.02	24.4 ± 3.2	48.1 ± 4.2
19	6	0.58 ± 0.04	22.2 ± 2.5	48.0 ± 4.5
21	7	0.47 ± 0.02	26.1 ± 3.2	48.3 ± 4.2

LV = left ventricular.
* Measured at thinnest point.
† Mean ± standard error.

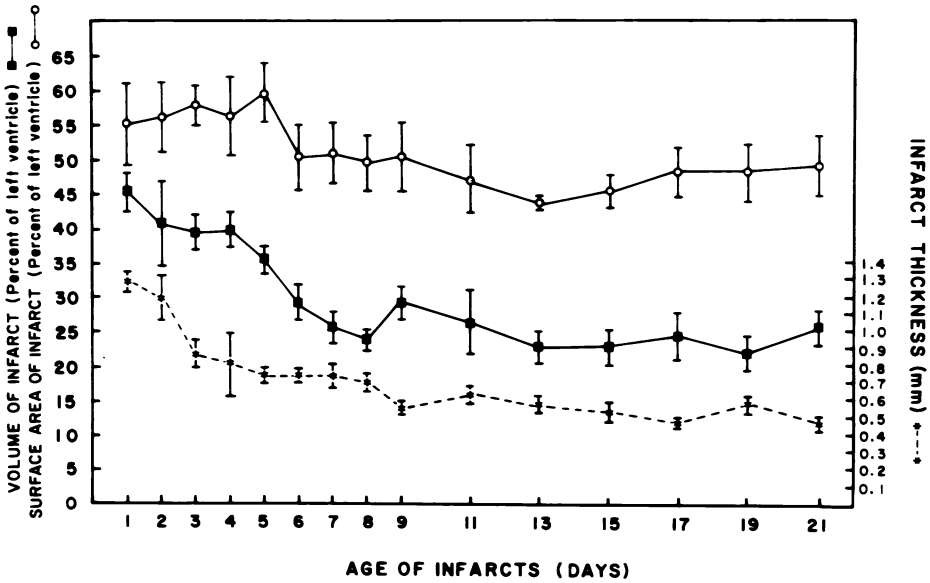
= 5 rats) at Day 1 to 26.1 ± 3.2% (N = 7 rats) at Day 21 ($P < 0.025$). In spite of the marked loss in volume of the infarcted tissue, there was no change in the surface area of the infarcted wall which involved 55.1 ± 7.2% of the surface area of the ventricle at Day 1 and 48.3 ± 4.2% at Day 21 (not significant, $P > 0.2$). Thus, throughout the evolution of the MI there was progressive thinning of the infarcted wall with reduction in the volume of the MI but no decrease in the percentage of the surface area of the left ventricle involved. Most of the loss of volume of infarcted tissue occurred during the first week (Text-figure 1).

Discussion

Ligation of the left coronary artery of the rat results in a reproducible sequence of myocardial changes, which is similar to that in man [26] but which evolves faster. This more rapid evolution may be related to the rat's smaller body size and higher rate of metabolism.[27] Wavy fibers were present in 100% of MI (13 of 13) less than 72 hours old. These thinned, wavy fibers, described in man by Bouchardy and Majno,[28] are thought to result from the stretching of noncontractile fibers during bulging of the ischemic tissue during systole.[29] The occurrence of wavy fibers in all rats killed early after coronary occlusion supports the view that they are related to ischemia and that this finding serves as a useful criterion to aid in the recognition of early MI.

Contraction bands [30] often were observed in cells at the margin of the early MI. These abnormal bands are composed of compressed adjacent sarcomeres which probably represent spasm of muscle fibers. Kloner et al [31] have shown that in the dog after 40 minutes of coronary occlusion as little as 2 minutes of reflow results in the appearance of numerous contraction bands in the ischemic myocardium to which flow has been restored. Flow studies in dogs, using microsphere techniques, have demonstrated that after coronary artery occlusion there is more blood flow at the edge of the ischemic zone than before occlusion.[32] Thus, the finding of contraction bands following permanent occlusion suggests that some reflow at the edge of rat infarcts may be occurring through the collateral circulation. An alternative explanation is that these contraction bands occur in areas of decreased but not absent collateral flow, which persists after coronary artery occlusion.[28]

In the rat the initial PMN response is not only shorter but also much less pronounced than in man. It is unclear why this response is so poor following an ischemic insult to the myocardium, as other forms of tissue injury in the rat, such as thermal injury to the skin, do result in a heavy PMN infiltrate.[33,34] This lack of PMN infiltration also was observed by Selye et al [14] and represents one major difference between naturally



TEXT-FIGURE 1—Quantitative changes in the evolution of myocardial infarction. Note that from Day 1 to Day 21 infarcted wall thickness and infarct volume decrease. Infarct surface area changes insignificantly, indicating that the decrease in the volume of the infarcted tissue is primarily a result of thinning of the wall.

occurring MI in man and surgically induced MI in rats. However, following this unusually brief and mild PMN response, the healing of the infarct proceeds as in man. Chronic inflammatory cells appear by Day 2 and then decrease in prominence as connective tissue proliferation increases. Healing is usually complete by Day 21.

Throughout the healing process the infarcted wall gradually thins as resorption of necrotic muscle progresses. The area of the infarcts, which is an estimate of the volume of infarcted tissue, decreased from a mean of 45.9 ± 5.9% at Day 1 to 26.1 ± 3.2% at Day 21; thus the volume of the scar which formed represented only approximately 50% of the volume of myocardium initially infarcted. The percentage of the surface area of the left ventricle involved changed insignificantly, indicating that the majority of tissue loss occurred as a result of the thinning of the infarcts.

In recent years there has been great interest in identifying interventions which might reduce MI size after coronary occlusion [1-10,17-21] and in determining the morphologic basis of the mechanism of action of such interventions.[35,36] Although most experimental studies have been in dogs, recent studies have shown that the rat model has great promise. Experiments using rats [17-21,37] have confirmed by direct measurements indirect observations in dogs,[1-4,8,9] and one drug, hyaluronidase, which reduces infarct size in the rat,[19] has been found to be beneficial in man as well.[10] The information derived from this qualitative and quantitative study of myocardial infarcts in rats provides a baseline for future studies using this model.

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Acknowledgments

The authors gratefully acknowledge the expert technical assistance of Carol Hare and Joseph Gannon.

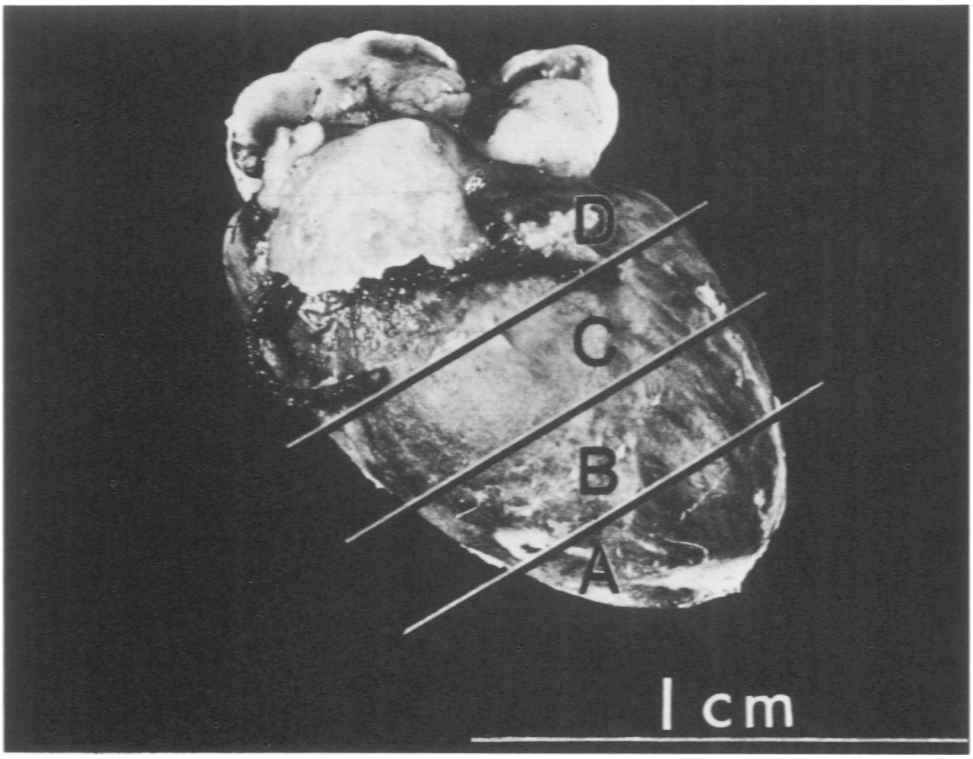


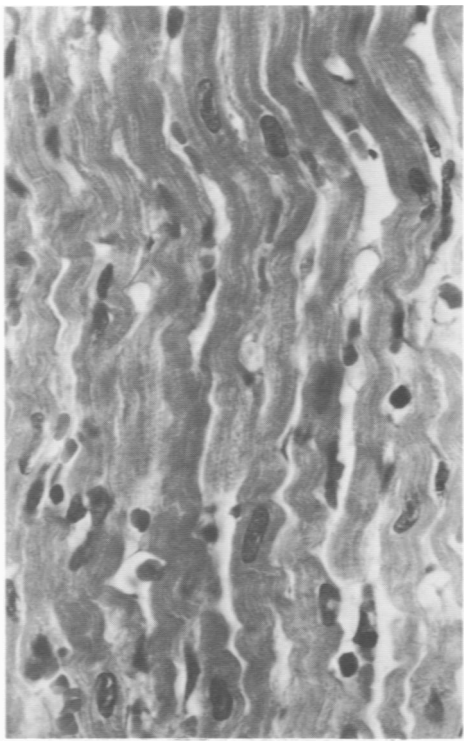
Figure 1—Gross photograph of rat heart after coronary artery occlusion. The parallel lines show the location of cuts made to divide the heart into four slices (*A,B,C,D,*).

Figure 2—Wavy fibers from the edge of a 24-hr-old infarct. Wavy fibers were a constant feature at the edge of the infarcts. (H & E, original magnification × 160)

Figure 3—Contraction bands (*arrow*) at edge of 24-hr-old infarct. These bands, which are fused sarcomeres presumably in fibers with spasm, were common at the edge of the infarcts and often adjacent to wavy fibers. (H & E, original magnification × 160)

Figure 4—Anitschkow cells (*arrow*) in a 7-day-old infarct. Such cells, not usually prominent in human infarcts, were present from Day 7 to Day 21 in areas of fibroblastic proliferation. (H & E, original magnification × 160)

Figure 5—Two-day-old infarct. Note necrotic fibers (*N*) and abundant granules representing calcium deposition (*arrows*). Calcium deposition was a prominent feature in the rat infarcts. (von Kossa, original magnification × 40) **Inset**—Calcification of an individual myocardial fiber (*arrow*) which has elicited a giant-cell (*G*) reaction in a 7-day-old infarct. (von Kossa, original magnification × 160)



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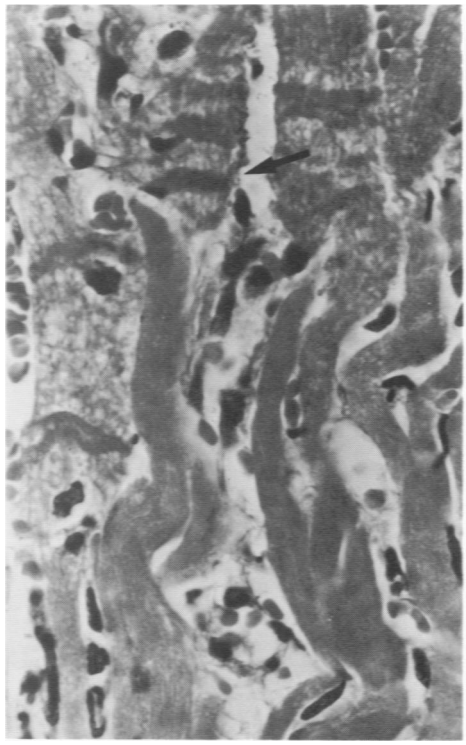

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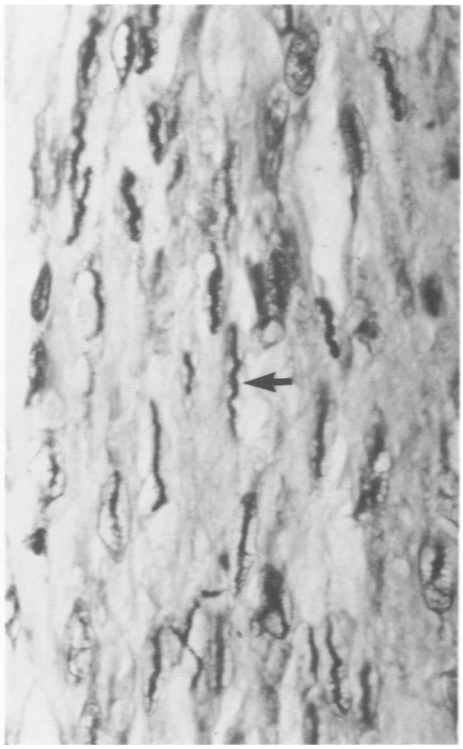

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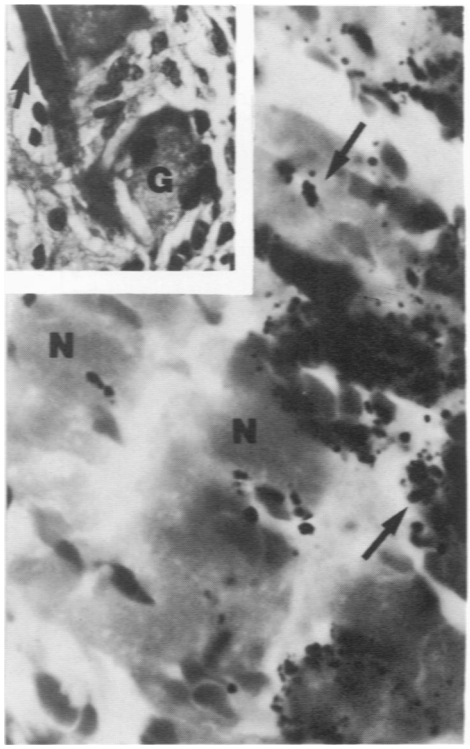

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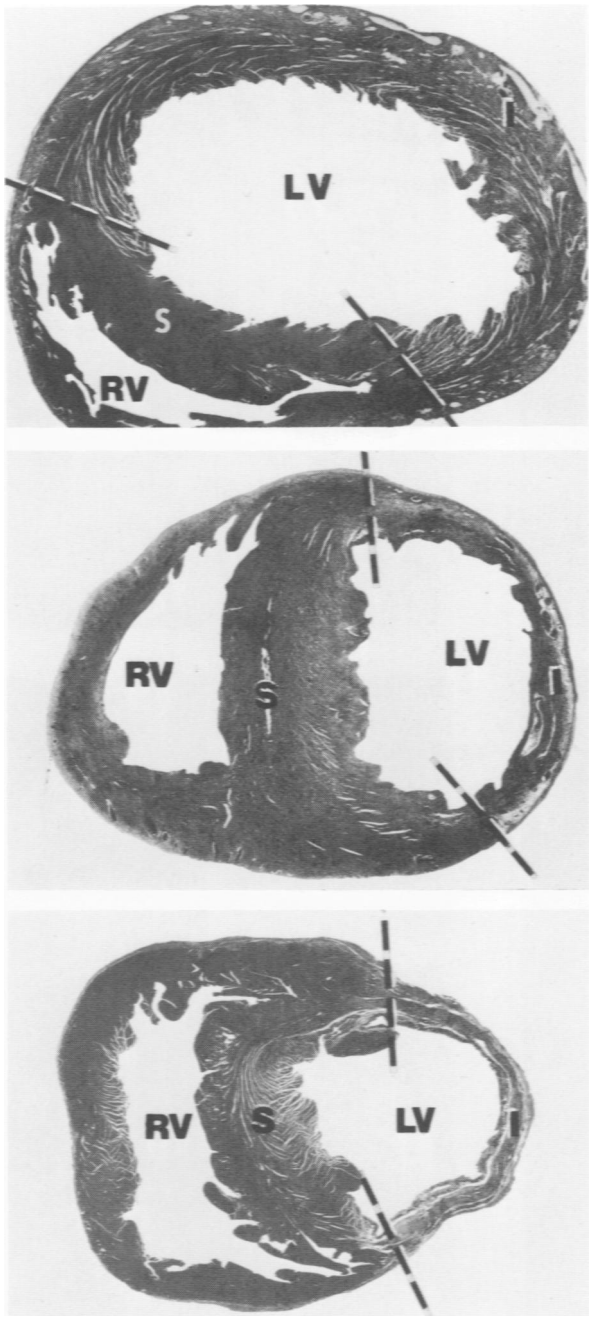

Figure 6—Quantitative changes during the healing of myocardial infarction. Shown are representative histologic sections from infarcts (*I*) 1 day old (*top*), 4 days old (*middle*), and 21 days old (*bottom*). During the evolution of myocardial infarction there are marked quantitative changes occurring in the heart: a) there is progressive thinning of the infarct (bounded by *broken lines*) and b) as the infarcted wall thins there is a decrease in the percentage of left ventricular (*LV*) tissue involved in the infarct (*RV* = right ventricle; S = septum). (H & E, original magnification × 9).