**Myocardial Infarction in Rats**

**Infarct Size, Myocyte Hypertrophy, and Capillary Growth**

Piero Anversa, Cesare Beghi, Yutaka Kikkawa, and Giorgio Olivetti

*From the Department of Pathology, New York Medical College, Valhalla, New York, and the Department of Pathology, University of Parma, Parma, Italy*

**SUMMARY.** To determine the compensatory reserve capacity of the ventricular myocardium following infarction, the left coronary artery in rats was ligated, and the animals were killed 40 days later. Infarcts affecting an average 23% of the left ventricle were characterized by a 27% hypertrophic growth of the remaining myocardium that produced a complete replacement of the necrotic tissue. In contrast, infarcts with an average 50% loss of mass resulted in 83% expansion of the spared myocardium that was inadequate for a complete restoration of ventricular tissue. Myocyte hypertrophy was 26% and 78% in small and large infarcts, respectively. Cellular hypertrophy in both cases involved significant increases in myocyte transverse area and myocyte length. After large infarcts, there was an 18% reduction in capillary surface and a 16% increase in the diffusion distance. Corresponding values for small infarcts were —10% and 9%. These alterations combined with the deficient reconstitution of myocardial mass following large infarcts resulted in 25%, 29%, and 30% deficits in the absolute amounts of capillary lumen, surface, and length per ventricle respectively. Even with small infarcts, a deficit was seen in capillary luminal surface (—16%), and length (—19%). In conclusion, we have demonstrated that cardiac hypertrophy following myocardial infarction is consistent with cellular shape changes characteristic of a combination of concentric and eccentric hypertrophic growth. However, cardiac muscle cells appear to be unable to compensate for the loss of mass induced by a 50% infarct. The inadequate adaptation of the capillary vasculature in the infarcted hearts suggests that the injured ventricle is more vulnerable to additional ischemic episodes. *(Circ Res 58: 26–37, 1986)*

**INFARCT size is an important determinant of prognosis in humans.** A certain threshold of infarct size seems to be the major cause of impairment of ventricular function in the early phase of the disease (Sobel, 1976). Quantitative studies have indicated that massive infarcts acutely affecting 40% or more of the left ventricular mass are associated with intractable cardiogenic shock (Page et al., 1971; Caulfield et al., 1976). In contrast, patients with relatively small infarcts often retain normal hemodynamic parameters (Kupper et al., 1977). Subsequent recovery of cardiac function is due to the regenerative capacity of the remaining noninfarcted portion of the ventricle. A significant unanswered question is how infarct size relates to the magnitude of tissue and myocyte hypertrophy that can be achieved by the surviving part of ventricular myocardium. The limit of this hypertrophic response, i.e., the maximal growth reserve of adult myocardium, has yet to be identified. Furthermore, it remains to be determined whether this kind of reactive hypertrophy represents a response to a pressure or a volume overload, or both (Sonnenblick et al., 1983). Another relevant issue to be resolved is whether hypertrophy of the spared myocardium is accompanied by a proportional or disproportional growth adaptation of the capillary bed. Reconstitution of cardiac mass with a sufficient oxygen supply to the cells may be assumed to improve the functional capacity of the ventricle, but an insufficient oxygen supply could contribute to its further deterioration.

To obtain data relevant to these unknown factors, the left coronary artery was ligated in rats to produce infarcts of different sizes, and the animals were killed 40 days later. Morphometric methodologies have been applied to investigate (1) the relative and absolute size of infarcts, (2) the relative and absolute volume of surviving myocardium, and the amount of its compensatory hypertrophic growth, (3) the average changes in myocyte size and shape, and in the subcellular components of myocytes, and (4) the average changes in the capillary structures controlling oxygen availability, diffusion, and transport within the myocardial tissue.

**Methods**

**Experimental Animals**

Ligation of the left coronary artery was performed in 45 male Wistar Kyoto rats at 80 days of age (Charles River Breeding Labs), by a technique described in detail elsewhere (Selye et al., 1960; Anversa et al., 1985a, 1985b). Twenty-two rats died shortly after the operation, mostly because of pulmonary edema. The remaining 23 animals were killed 40 days later (body weight 315 ± 20 g).
Control Animals

Three control groups were obtained: (1) unoperated rats at 80 days of age (body weight 251 ± 2 g) to establish the characteristics of the heart at the time of surgery, (2) unoperated rats at 120 days (body weight 319 ± 35 g) to establish the normal physiological growth of the heart during the 40-day period of investigation, and (3) sham-operated rats at 120 days (body weight 315 ± 24 g) to establish the influence of surgery on the growth of the heart. These groups consisted of 8, 6, and 16 animals, respectively. Sham operation was performed by placing an incomplete ligature around the coronary artery.

Fixation Procedure

Just before sacrifice, all animals were anesthetized with sodium pentobarbital (Nembutal, Abbott Laboratories). 3 mg/100 g body weight, ip and after abdominal aorta was isolated, a polyethylene cannula was inserted into the aorta and sealed in place with a ligature. The cannula was attached to a perfusion apparatus. In rapid succession, the heart was arrested in diastole by injecting approximately 1 ml of 1 M KCl through the jugular vein, the thorax was opened, perfusion with pH 7.2 phosphate buffer was started, and the right atrium was cut to allow the drainage of blood and perfusate. Perfusion pressure was adjusted at 85 mm Hg, nearly equal to the average diastolic pressure in adult anesthetized rats (Lais et al., 1977). After perfusion with buffer for 3 minutes, the coronary vasculature was perfused for 15 minutes with a solution containing 2% paraformaldehyde and 2.5% glutaraldehyde. The total osmolality of the fixative was 750 mOsM (Anversa et al., 1982). Subsequently, the heart was excised and the weights of the left ventricle, including the septum and the right ventricle, recorded. The volume of left ventricular myocardium was then determined by dividing its weight by the specific gravity of muscle tissue, 1.060 g/ml (Mendez and Keys, 1960).

Tissue Sampling

In the infarcted animals, the whole left ventricle was serially sliced into 2-mm-thick rings perpendicular to the axis of the heart from the apex to the base. All but one of the individually numbered slices of the left ventricle were flat embedded in glycol methacrylate so that the basal side was exposed for sectioning. The middle slice was cut radially to obtain approximately 15 1-mm-thick tissue blocks from the septum and adjacent regions of the free wall for electron microscopy. In the control groups, the mid-zone of the left ventricle and septum was sampled in a similar manner. This method of sampling was followed in order to obtain a uniform reference point, because the infarcts in this study were transmural, and some involved most of the free wall of the left ventricle (Fig 1, a and b). The specimens for electron microscopy were postfixed in a similar manner. This method of sampling was followed in order to obtain a uniform reference point, because the infarcts in this study were transmural, and some involved most of the free wall of the left ventricle (Fig 1, a and b). The specimens for electron microscopy were postfixed in 1% Porter-Blum microtome, and were stained with methylene blue and safranin. Morphometric sampling at a magnification of 630X consisted of counting the total number of myocyte nuclear profiles, N(n), in a measured area, A, of tissue sections in which cardiac muscle fibers were sectioned transversely. A square uncompressed tissue area equal to 21,609 μm² was delineated in the microscopic field by an ocular reticle (#105844, Wild Heerbrugg Instruments, Inc.). A total of 24 such fields were evaluated in each animal to determine the mean number of nuclear profiles per unit area of myocardium, N(n)μ.

Average nuclear length, D(n), was determined in each animal from 50 measurements made at a magnification of 1250X in longitudinally oriented myocytes viewed with a microscope with an ocular micrometer accurate to 0.5 μm. Five blocks were cut from each animal with myofibers sectioned parallel to their length to avoid longitudinal compression. Sections approximately 2 μm thick were collected and stained, and 10 measurements of nuclear length were recorded from each tissue section. Only those nuclei were measured in which the nuclear envelope was sharply defined at both ends and clusters of mitochondria were clearly visible in the areas adjacent to the nuclear edges.

Measurements of the number of myocyte nuclei per unit volume of myocardium, N(n)μ, were obtained using the equation (Loud and Anversa, 1984):

\[ N(n)μ = N(n)μ / \bar{D}(n). \]  

The total number of nuclei in each ventricle, N(n)μ, then was computed from N(n)μ and the corresponding total ventricular volume of viable myocardium, Vμ, previously determined:

\[ N(n)μ = N(n)μ × \bar{V}(μ) / N(n)μ. \]  

The volume fraction of myocytes in the myocardium, V(m),, was determined by low power electron microscopy (see below). Total ventricular volume, Vμ, multiplied by the volume fraction of myocytes, V(m),, and divided by the total number of myocyte nuclei in the ventricle, N(n)μ, yields the average myocyte cell volume per nucleus, V(m),, in each animal:

\[ \bar{V}(μ) = V(μ) × V(m) / N(n)μ. \]  

Electron Microscopic Morphometry

Six Araldite-embedded tissue blocks from each ventricle of each animal were trimmed for thin sectioning to obtain four areas of tissue with transversely sectioned myofibers and two with longitudinally oriented cells near the center of the ventricular wall. Low power electron micrographs
of transverse sections, five from each tissue block, were collected and printed at 5300X, calibrated with a diffraction grating replica magnification standard (E. F. Fullam, Inc.). These micrographs were analyzed morphometrically with a superimposed grid consisting of 140 sampling points and 14 test-line segments each 150 mm long.

Quantitative measurements consisted of point counts to determine the fractional volumes of myocardium composed of myocytes, capillary lumen, and other interstitial structures. The numbers of capillary and myocyte profiles in the sampled area were counted to estimate their numerical densities, average cross-sectional areas, and lengths per unit volume. Mean myocyte length per nucleus was derived from the quotient of mean cell volume per nucleus and average cross-sectional area. Sampling lines in the morphometric grid were used to determine capillary luminal surface. This luminal surface area is expressed relative to the volume of myocytes, not only because these cells constitute the major oxygen-consuming portion of the tissue, but also to eliminate the effects of possible variations in the interstitial space (Hoppeler et al., 1981). An identical number of high power electron micrographs, collected in a similar manner, were printed at 30,000X and utilized to evaluate the volume fractions of mitochondria, myofibrils, and matrix in the myocyte cytoplasm. An additional sampling of four random fields representative of longitudinal sections of myocyte cytoplasm were collected from each of two tissue blocks with longitudinally oriented myofibers, and printed at a final magnification of 15,000X. Mean sarcomere length in myofibrils was obtained from 50 measurements in each ventricle of each rat, using those sections that had been cut perpendicular to the fiber axis to avoid compression artifacts.

Data Collection and Analysis

All morphometric data were coded and the code broken only at the end of the experiment. Standard morphometric relationships and correction factors (Loud and Anversa, 1984) were applied in the computations of numerical density, size, and surface area of tissue components. The average distance from the capillary wall to the surrounding tissue was calculated following the methodology described by Hoppeler et al. (1981). This diffusion distance for oxygen can be measured approximately using the radius of a cylinder of muscle tissue supplied by the average capillary, based on the Krogh’s cylinder model for gas exchange in tissue (Weibel, 1979; Hoppeler et al., 1981), minus the mean radius of the capillary (Anversa et al., 1983b). Absolute volumes, surface areas, and lengths of component structures were evaluated from the products of total ventricular myocardial volume and their respective values per unit volume (Olivetti et al., 1980). Results are presented as means ± SD computed from the average measurements obtained from each rat. Statistical significance in multiple comparisons among independent groups of data, in which analysis of variance and the F-test indicated the presence of significant differences, was determined by the Bonferroni method (Wallenstein et al., 1980). P values < 0.05 were considered to be significant.

Results

Infarcted Animals

Forty days after surgery, the scarred tissue was seen to comprise different portions of the left ventricle (Fig. 1, a and b). Figure 2 shows that there was considerable variability in the total ventricular vol-

![Figure 1](http://circres.ahajournals.org/)

**Figure 1.** Sections of large methacrylate-embedded tissue slices of the left ventricle showing two transmural infarcts of different size (1a and 1b). Hematoxylin and eosin, 7X.
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FIGURE 2. Bars show the total left ventricular volume in each of the 23 infarcted animals. The shaded area in each bar shows the volume of surviving myocardium 40 days after surgery. The volume of infarcted tissue in each animal is represented by the clear area at the top of each bar with its volume % indicated numerically.

FIGURE 3. Graphical representation of the total number of myocyte nuclei vs. the volume % of scarred tissue in each infarcted ventricle of the 23 animals studied. The data, represented by the linear regression line (slope = -0.91; intercept = 31.7), have a statistically significant correlation coefficient (r = 0.89; P < 0.001).

FIGURE 4. Plot of mean cell volume per nucleus vs. the percent of scarred tissue in each of the 23 infarcted ventricles examined. The data, represented by the linear regression line (slope = 894.7; intercept = 19,695), have a statistically significant correlation coefficient (r = 0.90; P < 0.001).

umes and in the volumes of viable and scarred tissues among these animals. The volume percent of infarcts was found to range from 1.3% to 21.3% of the whole ventricle.

The number of myocyte nuclei remains fixed in the left ventricular myocardium of normal adult rats (for review, see Rakusan, 1984). Thus, any loss of cardiac mass from ischemic necrosis results in a proportional loss of myocyte nuclei and, subsequently, a proportional increase in the amount of connective tissue scar in the ventricle. Further, the initial volume of spared tissue is proportional to the number of myocyte nuclei remaining in the ventricular myocardium. Figure 3 shows the graphical comparison of the percent of scarred tissue in the whole ventricle vs. the total number of myocyte nuclei measured in the spared myocardium of each heart. The regression line demonstrates a significant (r = 0.89; P < 0.001) negative slope indicating that smaller residual numbers of myocyte nuclei are associated with larger infarcts.

To test whether infarct size is related to myocyte...
hypertrophy, the mean cell volume per nucleus in each heart was plotted vs. the percent of scarred tissue in the ventricle. Figure 4 represents these data which show a highly significant positive correlation \( r = 0.90; \ P < 0.0001 \). This relationship demonstrates that larger infarcts are associated with greater cell volumes in the spared portion of the myocardium.

**Adaptation of the Heart after Myocardial Infarction**

As stated in the introduction, one of the major objectives of the present study was to establish whether the magnitude of tissue regeneration following myocardial infarction leads to a complete or incomplete reconstitution of myocardial mass in relation to infarct size. Since it is impossible at present to measure absolute infarct size and the actual amount of tissue growth in each individual case, the 23 infarcted animals were divided into two groups that were arbitrarily called animals with small and large infarcts, respectively. This subdivision was also suggested by the graphic distribution of values plotted in Figure 4. The first group of 16 animals included infarcts comprising up to 12% of the ventricle, and the second group of seven animals included infarcts involving more than 12% of the ventricular wall.

The changes in weight of the heart, left ventricle, and right ventricle during growth and after myocardial infarction are illustrated in Figure 5. In comparison with sham-operated controls, myocardial infarction in both experimental groups produced practically no change in the weight of the left ventricle. The maintenance of a constant left ventricular weight despite the presence of scarred tissue implies that a significant amount of tissue hypertrophy has occurred in the spared myocardium. Figure 5 also shows that from 80–120 days there was a comparable increase in the weight of the left ventricle, 29% and 25%, in unoperated and sham-operated animals, suggesting that the operation had no effect on the growth of the heart.

**Infarct Size and Hypertrophy of the Surviving Myocardium**

The histometric measurement of infarct size (Fig. 2), based on the computation of the fractional volume of connective tissue in the ventricle, does not take into account the volume changes that occurred in the infarcted and noninfarcted regions of the heart throughout the 40-day period of this study. Hypertrophy takes place in the spared myocardium, and shrinkage of the necrotic zone develops progressively with time. These alterations lead to an underestimation of the extent of myocardial infarction and to an overestimation of the initial volume of ventricular mass destined to survive the ischemic event. Since these volume changes cannot be predicted with reasonable accuracy, a recently developed morphometric methodology (Anversa et al., 1985a, 1985b), independent of such unknown factors, was used in the present study.

Table 1 shows the primary measurements utilized for the determination of real infarct size (i.e., the size prior to shrinkage) and of the magnitudes of hypertrophic growth that have occurred in the surviving myocardium and its myocyte population. The computation of the aggregate number of myocyte nuclei in the left ventricle demonstrated that, in comparison with unoperated control rats at 80 days, animals with small infarcts had an average 23% loss of myocyte nuclei. A similar calculation revealed a 50% loss in animals with large infarcts. When the two infarct groups were compared, rats with larger infarct size exhibited a 35% greater loss of myocyte nuclei. The effect of the surgical procedure on nuclear density, length, and total number was also

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**Figure 5.** Gross cardiac changes produced by normal and postoperative growth, and myocardial infarction. Values are means ± s.e. Group 1 = unoperated rats at 80 days of age; group 2 = unoperated rats at 120 days; group 3 = sham-operated rats at 120 days; group 4 = rats with small infarcts; group 5 = rats with large infarcts. * Indicates a statistically significant difference, \( P < 0.05 \), vs. group 1.
examined. As expected, no difference was found between 120-day-old unoperated controls and sham-operated controls of the same age.

Table 2 shows the computations of absolute infarct size and of the overall growth adaptation that has occurred in the surviving myocardium after small and large infarcts of the left ventricle. By dividing the number of myocyte nuclei still present in the viable myocardium of the two infarcted animal groups by the corresponding value in the unoperated control group at 80 days (Table 1), the percentages of nuclei spared and the standard deviations were calculated (Anversa et al., 1978). This percentage is 77.1 ± 16.4 and 22.9 ± 16.4 for small infarcts, and 49.8 ± 10.1 and 50.2 ± 10.1 for large infarcts. Therefore, small infarcts averaged 23% of the whole ventricle, and large infarcts, 50%. The products of the percentages of nuclei lost and spared and the mean volume of myocardium in the control left ventricle (unoperated animals at 80 days) described the subdivision of the left ventricle into two parts: the volume of myocardium destined to become necrotic as a result of coronary artery occlusion (infarcted myocardium) and the volume of myocardium destined to survive (spared myocardium), listed in Table 2. In addition, utilizing the same computations and the volume of myocardium in sham-operated controls, the expansion of the spared myocardium from 80–120 days of age was calculated.

When these data were compared with the volumes of infarcted and surviving myocardium in the experimental groups, postoperative tissue growth (POG) and hypertrophic growth (HG), as well as the magnitude of shrinkage of the infarcted tissue, could be evaluated (Table 2). It can be seen that in small infarcts the spared myocardium has expanded 55%, 27% as a result of HG and 22% as a consequence of POG. The growth response of the viable myocardium was sufficient to reconstitute an adequate amount of cardiac mass, since no statistically significant difference was found between the volumes of myocardium in sham-operated (807 ± 63 mm³) and infarcted (772 ± 61 mm³) animals. In large infarcts, the volume of the spared myocardium increased by 124%. HG and POG accounted for 83% and 22%, respectively. The addition of myocardial mass in large infarcts was inadequate, −11%.
for a complete reconstitution of viable tissue. The infarcted tissue has undergone approximately a 59% loss of volume during the 40-day period in both infarct groups.

**Infarct Size and Myocyte Cell Volume per Nucleus**

Average myocyte volume per nucleus was determined directly in each of the five groups of animals following the morphometric procedure described in Methods. Figure 6 shows the dimensional characteristics of left ventricular myocytes in control and infarcted animals. By comparing the value obtained in rats with small infarcts with those measured in the unoperated controls at 80 days and sham-operated controls at 120 days, it was seen that the 61% overall increase in cell size was the result of a 26% HG and 27% POG. After large infarcts of the ventricle, the cellular response was 126%, 78% by HG and 27% by POG. Finally, mean cell volume per nucleus in this group was 41% greater than that in animals with small infarcts. It cannot be excluded, however, that some variation in sampling between the two groups of infarcts may have influenced our morphometric determinations.

**Adaptations of Myocytes and Capillaries**

Figure 7 shows that the volume fraction of myocytes in the myocardium was nearly constant in all five groups of animals. In comparison with group 1, animals with small and large infarcts exhibited a 19% and 40% increase in myocyte transverse cross-sectional area (MCSA), respectively. These changes were the result of a 6% POG, and a 12% and 32% HG. Rats with large infarcts had a MCSA 18% greater than that of animals with small infarcts. Since the volume percent of myocytes in infarcted left ventricles remained constant, the increases in MCSA were accompanied by approximately equiv-

![Cell Volume per Nucleus, \(\mu m^3\)](image)

**Figure 6.** Values are means ± so. Group 1 = unoperated rats at 80 days; group 2 = unoperated rats at 120 days; group 3 = sham-operated rats at 120 days; group 4 = rats with small infarcts; group 5 = rats with large infarcts. * Indicates a statistically significant difference, P < 0.05, vs. group 1. ** Indicates a statistically significant difference vs. group 1. *** Indicates a statistically significant difference between groups 4 and 5.

![Volume Percent of Myocytes](image)

![Transverse Cross Sectional Area of Myocytes, \(\mu m^2\)](image)

![Number of Myocyte Profiles per mm² of Myocardium](image)

**Figure 7.** See legend to Figure 6.

![Myocyte Length per Nucleus, \(\mu m\)](image)

**Figure 8.** See legend to Figure 6.

alent reductions in the numerical density of these cells per unit area of myocardium (MND).

From the values of mean myocyte cell volume per nucleus and average cross-sectional area, mean myocyte length per nucleus was derived (Fig. 8). It can be seen that, in animals with small infarcts, from 80–120 days old, there was a 37% lengthening of the average cell. POG and HG contributed 19% and 15%, respectively. In animals with large infarcts, the increase in cell length per nucleus was 64%, and since POG was identical to that in animals with
small infarcts, HG contributed for 38%. Measurements of sarcomere length were found to be practically identical in all animal groups, ranging from 2.01-2.07 μm.

The volume fraction of capillary lumen in the myocardium, transverse luminal area of capillary profiles (CCSA), and capillary numerical density (CND) are shown in Figure 9. With respect to group 3, CND significantly decreased by 15% and 22% in small and large infarcts as a result of HG. Because of the variability of the mean values, the changes in the volume percent of capillary lumen and CCSA were found not to be statistically significant.

Relationships between the myocyte population and its capillary supply in the ventricle are illustrated in Figure 10. When the infarcted animals, groups 4 and 5, are compared with sham-operated controls, group 3, a 10% and 18% decrease in capillary luminal surface area was observed as a consequence of HG. However, only the latter change was statistically significant. Because of the increases in MCSA, the diffusion distance for oxygen increased 9% and 16% in small and large infarcts, respectively. The ratio of capillary profiles-to-myocyte profiles was not altered in any animal group.

Figure 11 shows the volume percentages of mitochondria and myofibrils in myocyte cytoplasm. No change occurred in these values after small infarcts. However, in comparison with sham-operated controls, large infarcts demonstrated an 11% decrease in the volume fraction of the mitochondrial compartment and a 12% increase in the myofibrillar component of the cells. These changes produced a corresponding 20% reduction of the mitochondria:myofibrils volume ratio.

Table 3 shows the amount of growth that was achieved over a period of 40 days following the induction of infarcts affecting an average 23% and 50% of the left ventricle, and the amount of tissue growth needed for a complete restoration of muscle mass. It can be seen that increases in capillary luminal volume and surface, and total capillary length were less than the overall growth of myocytes in both infarcts groups. Although HG produced a striking growth of myocardial components, a substantial residual deficit was found for myocyte volume and capillary luminal volume, surface, and length. Furthermore, the deficits were relatively greater for capillaries than for myocytes.

Discussion

Infarct Size and Myocardial Hypertrophy

The findings of the present study indicate that myocardial infarction evokes a hypertrophic re-
response in the surviving tissue that varies with the extent of the initial myocardial damage. Infarcts affecting an average 23% of the left ventricle are characterized by a 27% increase in volume of the remaining functioning myocardium that results in a practically complete reconstitution of the original myocardial tissue. In contrast, infarcts with an average 50% loss of contractile mass are associated with an 83% expansion of the spared myocardium that is inadequate for a full restoration of ventricular tissue. Myocardial hypertrophy in the rat, induced by different experimental procedures, consists at the most of an increase in weight of the left ventricle of nearly 80% (Nair et al., 1968; Anversa et al., 1978, 1980b; Hatt et al., 1979). Thus, a 50% infarct appears to produce a sufficiently large stress on the spared myocardium to stimulate its maximum hypertrophic capacity. It follows that infarcts of this size may constitute an upper limit beyond which greater destruction of cardiac mass cannot be compensated by the remaining myocardium and becomes incompatible with survival.

Recent studies have shown that rats with relatively small infarcts, comprising up to 30% of the left ventricle, have no detectable impairment of cardiac function. However, rats with infarcts affecting more than 46% of the ventricle exhibit congestive heart failure (Pfeffer et al., 1979). The present results, demonstrating that infarcts in these same size ranges are associated, respectively, with complete or incomplete replacement of the necrotic tissue, may explain, at least in part, the alterations in ventricular performance measured physiologically.

Infarct Size and Myocyte Cell Volume and Shape

It is the current view that cellular growth in hypertrophy is proportional to the amount of stress imposed on the heart, although this hypothesis has never been demonstrated experimentally. On such a basis, it can be assumed that following myocardial infarction the extent of cellular enlargement in the surviving tissue would be proportional to the magnitude of myocardial cell loss. Consistent with this concept, 40 days after coronary artery ligation, mean cell volume per nucleus showed a highly significant positive correlation with infarct size, indicating that greater myocyte cell volumes accompany larger infarcts.

Following small and large infarcts of the left ventricle, hypertrophy of average myocyte cell volume per nucleus was 26% and 78%, respectively. Although large infarcts induced a 41% greater hypertrophy of cardiac muscle cells than small infarcts, the addition of contractile mass was found to be inadequate for a full reconstitution of functioning tissue. For a complete recovery of the myocyte compartment of the myocardium, mean myocyte volume per nucleus should have increased as much as 104% instead of 78%. Thus, cardiac muscle cells appear to be unable to offset by cellular hypertrophy alone the loss of mass induced by infarcts involving 50% of the ventricle. It remains to be determined, however, whether a greater stress on the heart may evoke myocyte hyperplasia as an additional compensatory mechanism. Several studies on human hearts have shown that myocyte proliferation occurs in both left and right ventricles in a variety of pathological states that impose a large and sustained overload on the myocardium (Linzbach, 1960; Astorri et al., 1971).

The dimensional changes of ventricular myocytes after myocardial infarction which involve increases in myocyte cross-sectional area and length are consistent with cellular shape changes characteristic of concentric and eccentric hypertrophy in the intact ventricular wall (Anversa et al., 1980a, 1980b, 1982). Loss of cardiac cells in the ventricle can be expected to result in a greater stress on the remaining viable myocytes. To reduce the magnitude of systolic stress, myocytes would tend to hypertrophy by increasing their diameter, as shown here and in previous observations (Turek et al., 1978; Rubin et al., 1983; Anversa et al., 1985b). On this basis, infarction-induced hypertrophy may be viewed, at least in
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Hypertrophy and Residual Deficit of Myocardial Structures Following Small and Large Infarcts of the Left Ventricle

<table>
<thead>
<tr>
<th>Volume (mm³)</th>
<th>Computed from animals</th>
<th>Measured in Infarcted animals at 120 days (3)</th>
<th>Sham-operated animals at 120 days (4)</th>
<th>% Change</th>
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</thead>
<tbody>
<tr>
<td>Myocytes</td>
<td>SI</td>
<td>401 ± 99</td>
<td>507 ± 108</td>
<td>634 ± 52</td>
</tr>
<tr>
<td></td>
<td>LI</td>
<td>259 ± 62</td>
<td>328 ± 67</td>
<td>585 ± 84</td>
</tr>
<tr>
<td>Capillary lumen</td>
<td>SI</td>
<td>42.8 ± 12.5</td>
<td>48.1 ± 14.3</td>
<td>61.0 ± 16.5</td>
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<tr>
<td></td>
<td>LI</td>
<td>27.6 ± 7.9</td>
<td>31.1 ± 9.1</td>
<td>48.1 ± 10.5</td>
</tr>
<tr>
<td>Surface area (mm²), capillary lumen</td>
<td>SI</td>
<td>34,634 ± 9,899</td>
<td>40,520 ± 9,715</td>
<td>45,453 ± 6,721</td>
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<tr>
<td></td>
<td>LI</td>
<td>22,369 ± 6,244</td>
<td>26,191 ± 6,070</td>
<td>38,090 ± 6,375</td>
</tr>
<tr>
<td>Length (m), capillaries</td>
<td>SI</td>
<td>1,920 ± 535</td>
<td>2,378 ± 610</td>
<td>2,554 ± 254</td>
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<tr>
<td></td>
<td>LI</td>
<td>1,240 ± 337</td>
<td>1,537 ± 384</td>
<td>2,210 ± 398</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD. SI = small infarcts; LI = large infarcts. POG + HG = values indicating the combined effect of postoperative tissue growth (POG) and hypertrophic growth (HG) on the surviving myocardium. HG = values indicating the effect of hypertrophic growth alone on the surviving myocardium. RD = values indicating the residual deficit of myocardial components 40 days after coronary artery ligation.

* Indicates a percent change that is statistically significant, P < 0.05.

Part, as pressure overload hypertrophy, despite the presence of a normal pressure (Sonnenblick et al., 1983).

Physiological studies performed over several weeks in the dog heart have shown a progressive increase of the end-diastolic segment lengths in the normal regions of infarcted ventricles (Theroux et al., 1977). Similar adaptations have been observed in chronic volume-overloaded left ventricles (Ross and McCullagh, 1972) in which lengthening of the myocyte population would have the effect of counteracting the greater end-diastolic wall stress by contributing to the enlargement in chamber volume as suggested by Grossman (1980). Therefore, cardiac hypertrophy associated with myocardial infarction appears to be the result of both pressure and volume overload, consonant with the increases in myocyte wall to the surrounding tissue (Weibel, 1979; Hoppeler et al., 1981). After large infarcts of the left ventricle, the hypertrophied surviving myocardium was characterized by an 18% reduction in capillary surface and a 16% increase in the diffusion distance for oxygen. In the presence of small infarcts, a 10% decrease in surface and a 9% increase in diffusion distance were demonstrated, although only the latter alteration was statistically significant. Therefore, a deficit in capillary adaptation exists following myocardial infarction, and the magnitude of this deficit depends on the extent of hypertrophy which is related to infarct size. A greater diffusion distance for oxygen has also been reported in the hypertrophied right ventricular myocardium 1 month after coronary occlusion (Turek et al., 1978; Anversa et al., 1984).

At the subcellular level, the composition of myocyte cytoplasm in left ventricular myocytes is not modified by the hypertrophic growth accompanying small infarcts of the ventricle. In contrast, a reduction of the mitochondria/myofibrils volume ratio was found in large infarcts, indicating a growth of these components disproportionate to each other and to the cell volume as a whole. Reduction of the mitochondria/myofibrils volume ratio is a consistent subcellular alteration occurring in myocytes as a result of pressure overload hypertrophy (Anversa et al., 1983b). Exercise hypertrophy, on the other hand, is characterized by the maintenance of a constant mitochondria/myofibrils ratio (Anversa et al., 1983a).

Capillary Adaptation

The response of the capillary vasculature has been analyzed by evaluating the structural parameters involved in the oxygenation of the myocardium, i.e., capillary luminal volume density, capillary luminal surface density, and the distance from the capillary wall to the surrounding tissue (Weibel, 1979; Hoppeler et al., 1981). After large infarcts of the left ventricle, the hypertrophied surviving myocardium was characterized by an 18% reduction in capillary surface and a 16% increase in the diffusion distance for oxygen. In the presence of small infarcts, a 10% decrease in surface and a 9% increase in diffusion distance were demonstrated, although only the latter alteration was statistically significant. Therefore, a deficit in capillary adaptation exists following myocardial infarction, and the magnitude of this deficit depends on the extent of hypertrophy which is related to infarct size. A greater diffusion distance for oxygen has also been reported in the hypertrophied right ventricular myocardium 1 month after coronary occlusion (Turek et al., 1978; Anversa et al., 1984).

Early hypertrophy in genetically determined hypertension has been found to be associated with an inadequate compensation of the capillary bed brought about through a relative decrease in CND with no change in capillary size (Tomanek et al., 1982). With aging, however, CND returns to control values, resulting in the restoration of a normal capillary luminal volume and surface density in the ventricular myocardium (Tomanek et al., 1982). A significant increase in intercapillary distance has also been reported in other models of induced hypertensive hypertrophy (Henquell et al., 1977). Volume
overload hypertrophy of the left and right ventricles has been described to be accompanied by either an adequate (McElroy et al., 1978; Anversa et al., 1983a) or inadequate (Rakusan et al., 1980; Anversa et al., 1982) growth adaptation of the capillary bed through changes in CND. These observations demonstrate that both concentric and eccentric hypertrophy may result in alterations of the structural properties of the microvasculature that lead to a reduced maximum oxygenation capacity per unit volume of tissue.

Ventricular Adaptation

Despite the conspicuous reconstitution of myocardium following large infarcts, an additional significant fraction of tissue still needs to be replaced in order to obtain a complete restoration of the left ventricular mass. Furthermore, it was demonstrated that 25%, 29%, and 30% deficits in the absolute amounts of capillary lumen, surface, and length were present in the infarcted ventricles. With small infarcts, the deficit per ventricle was markedly less, showing only a 16% and 19% lack in capillary luminal surface and length.

It has been repeatedly shown that the weight of the infarcted ventricle, when healing is completed, is not altered, despite the marked thinning of the wall in the scarred region, suggesting that tissue hypertrophy in the unaffected myocardium has been able to compensate for the loss in myocardial mass (Pfeffer et al., 1979; Rubin et al., 1983; Anversa et al., 1984). The present morphometric results demonstrate that caution should be taken in the interpretation of gross cardiac changes following myocardial infarction. Tissue loss even of a moderate degree leads to an insufficient response of the capillary bed that is much greater in the presence of infarcts involving as much as 50% of the whole ventricle.

Determination of Infarct Size

Current methodologies for the estimation of infarct size are based on the evaluation of the fraction of endocardial circumference or endocardial surface bordering the infarcted portion of the ventricle (Fishbein et al., 1978; Pfeffer et al., 1979; Fletcher et al., 1981). However, these histometric techniques contain various sources of error, dependent upon the progressive reduction of necrotic tissue with time, scar formation with thinning of the wall (Fishbein et al., 1978), dilation of the ventricular chamber (Fletcher et al., 1981; Hochman and Bulkey, 1982), and the unknown amount of hypertrophic growth of the spared myocardium. These are all dynamic processes that progressively change the proportion between viable and nonviable tissue in the injured ventricle. The morphometric approach developed in our laboratory (Anversa et al., 1985a, 1985b) and utilized in the present study has enabled the evaluation of (1) real infarct size, (2) magnitude of normal and induced tissue growth occurring in the spared myocardium, (3) extent of myocyte and capillary adaptation, and (4) residual deficits in capillary and myocyte growth.

By applying this methodology, we have shown that an approximate 59% shrinkage of the necrotic myocardium occurs in a 40-day period in both small and large infarcts (Table 2). Furthermore, by comparing the histometric method with the morphometric method, we found that the former technique results in an underestimation of real infarct size of approximately 70%. In fact, small infarcts measured planimetrically yield a myocardial loss of 6.73 ± 3.45% compared to 22.92 ± 16.42% measured morphometrically. Corresponding values in large infarcts are 16.48 ± 2.96% and 50.21 ± 10.14%.

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Address for reprints: Piero Anversa, M.D., Department of Pathology, New York Medical College, Valhalla, New York 10595.

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References


Caulfield JB, Leinbach R, Gold H (1976) The relationship of coronary bed that is much greater in the presence of infarcts involving as much as 50% of the whole ventricle.
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P Anversa, C Beghi, Y Kikkawa and G Olivetti

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