Cellular Basis of Chronic Ventricular Remodeling After Myocardial Infarction in Rats

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To determine whether the hypertrophic response of the surviving myocardium after infarction leads to normalization of ventricular hemodynamics and wall stress, the left coronary artery was ligated in rats. One month later, the rats were killed. In infarcts affecting an average 38% of the free wall of the left ventricle (small infarcts), reactive hypertrophy in the spared myocardium bordering and remote from the scar was documented by increases in myocyte cell volume per nucleus of 43% and 25%, respectively. These cellular enlargements resulted in a complete reconstitution of functioning tissue. However, left ventricular end-diastolic pressure was increased, left ventricular dP/dt was decreased, and diastolic wall stress was increased 2.4-fold. After infarctions resulting in a 60% loss of mass (large infarcts), myocyte hypertrophy was 81% and 32% in the regions adjacent to and distant from the scar, respectively. A 10% deficit was present in the recovery of viable myocardium. Functionally, ventricular performance was markedly depressed, and diastolic wall stress was increased ninefold. The alterations in loading of the spared myocardium were due to an increase in chamber volume and a decrease in the myocardial mass/chamber volume ratio that affected both infarct groups. Chamber dilation was the consequence of the combination of gross anatomic and cellular changes consisting, in the presence of small infarcts, of a 6% and a 19% increase in transverse midchamber diameter and in average myocyte length per nucleus, respectively. In the presence of large infarcts, transverse and longitudinal chamber diameters expanded by 27% and 11%, respectively, myocyte length per nucleus expanded by 26%, and the mural number of myocytes decreased by 10%. In conclusion, decompensated eccentric ventricular hypertrophy develops chronically after infarction, and growth processes in myocytes are inadequate for normalization of wall stress when myocyte loss involves nearly 40% or more of the cells of the left ventricular free wall. The persistence of elevated myocardial and cellular loads may sustain the progression of the disease state toward end-stage congestive heart failure. (Circulation Research 1991;68:856–869)

After acute myocardial infarction, pump function is reduced in direct proportion to the extent of myocardium that is lost on an obligatory basis; that is, ejection fraction falls as a function of infarct size.1,2 Infarcts affecting 40% or more of the left ventricle lead to severe myocardial dysfunction that may result in sudden cardiac death or acute congestive heart failure.3 In contrast, smaller infarcts may be initially associated with moderate alterations of hemodynamic parameters that may rapidly disappear during the evolution of the healing process. Since reactive hypertrophy of the unaffected ventricular mass is an early event that characterizes the myocardial response to infarction,4,5 the reconstitution of functioning tissue may play a major role in the recovery and maintenance of cardiac performance after ischemic myocardial injury. Compensatory growth mechanisms continue to occur chronically so that, through a progressive increase in size of the viable myocytes, most of the originally lost myocardium is replaced by fewer en-
larged ventricular cells.\textsuperscript{6,7} However, it is still unknown whether the regeneration of myocardial mass is capable of normalizing ventricular function or whether architectural changes such as ventricular dilation and thinning of the wall may complicate the long-term outcome of myocardial infarction\textsuperscript{8–11} by maintaining an elevated loading state in the injured ventricle. Moreover, it is unclear whether the cellular hypertrophic response in the regions bordering and remote from the infarct is adequate to restore cell stress within normal limits. Side-to-side slippage of cells within the wall can also increase stress at the myocyte level, as recently shown 48 hours after occlusion of the left coronary artery.\textsuperscript{12} The Laplace load on the remaining myocytes will be highly dependent on the anatomic rearrangements that govern the relation between wall thickness, number of cells in the wall, chamber radius, and resultant myocardial function.\textsuperscript{13–16} The inability of the heart to reestablish normal wall stress may be the critical factor in the evolution of ischemic cardiomyopathy after infarction. Therefore, the present study was undertaken to characterize the structural and functional properties of the infarcted heart at the completion of the healing process to identify how the remodeling of the ventricle interacts with cardiac hemodynamic parameters in the definition of the loading state of the myocardium after ischemic necrosis. An experimental protocol similar to that previously used in our laboratory was used in the present study, since a marked reactive hypertrophic response was shown to occur in the surviving myocardium approximately 1 month after coronary artery occlusion.\textsuperscript{6,7} However, it remained to be determined whether the reconstitution of myocardial mass resulted in a substantial recovery of ventricular pump performance and whether the increase in wall stress generated by the segmental loss of tissue had influenced the degree and pattern of myocyte growth. The nature, magnitude, and distribution of the mechanical overload, on the one hand, and the extent, characteristics, and heterogeneity in myocyte hypertrophy, on the other, constitute the functional and structural bases of chronic ventricular remodeling after infarction.

Materials and Methods

Experimental Animals

Ligation of the left coronary artery was performed in 55 male Wistar-Kyoto rats at 3 months of age (Charles River Breeding Laboratories, North Wilmington, Mass.) by use of a surgical procedure previously described.\textsuperscript{4} Briefly, with the rats under ether anesthesia, the thorax was opened, and the heart was exteriorized by applying a light pressure on the thorax. The left coronary artery was then ligated, the chest was closed, and the rats were allowed to recover. To reduce postoperative pain, 5 mg/kg morphine sulfate was administered subcutaneously. Twenty-seven rats died shortly after the operation, mostly because of pulmonary edema. The remaining 28 rats were killed a month later. Nineteen sham-operated rats were used as controls.

Functional Measurements

Before cardiac arrest was induced, the surviving 28 rats were anesthetized with 300 mg/kg i.p. chloral hydrate, and the external right carotid artery was exposed and cannulated with a microtip pressure transducer catheter (model PR 249, Millar Instruments, Houston) connected to an electrostatic chart recorder (model ES 1000B, Gould, Cleveland, Ohio). After the monitoring of arterial blood pressure, the catheter was advanced into the left ventricle for the evaluation of left ventricular pressures. Moreover, the rate of rise and fall of left ventricular pressure was derived by active analog differentiation of the pressure signal (differentiator amplifier No. 13-4615-71, Gould). Thus, measurements were made of systolic and diastolic arterial and left ventricular pressures and maximal dP/dt in the closed-chest preparation. These events were continuously monitored and inscribed on recording paper for subsequent analysis. No adjustments were made for individual variations in preload or afterload.

Perfusion Fixation

At the completion of the hemodynamic measurements, the abdomen was opened, and the abdominal aorta below the renal arteries was cannulated with a polyethylene catheter (PE-200) filled with phosphate buffer (0.2 M, pH 7.4) and heparin (100 IU/ml). The catheter was first sealed in place with a ligature and then connected to a perfusion apparatus. In rapid succession, the heart was arrested in diastole by an intravenous injection of approximately 1 ml KCl (1 meq/ml) through the jugular vein, the thorax was opened, perfusion with phosphate buffer was started, and the right atrium was cut to allow the drainage of blood and perfusate. Perfusion pressure was adjusted to diastolic arterial pressure measured in vivo. The left ventricular chamber was filled with fixative and kept at a pressure equal to approximately 0–5 mm Hg throughout the fixation procedure. After perfusion with buffer for 3 minutes, the coronary vasculature was perfused for 15 minutes with a glutaraldehyde-paraformaldehyde mixture diluted 1:1 with phosphate buffer.\textsuperscript{17} Subsequently, the heart was excised, and the weights of the left ventricle, including the septum, and the right ventricle were recorded. The volume of left ventricular myocardium was then determined by dividing its weight by the specific gravity of muscle tissue, 1.06 g/ml.\textsuperscript{17}

Although differences in left ventricular end-diastolic pressure were found between control and infarcted rats (see “Results”), all hearts were fixed at a constant intracavitary pressure. This was done to assess alterations in chamber size, wall thickness, myocyte length and diameter, and number of myocytes across the ventricular wall, which were directly related to infarct size independent from variations in filling pressure at the time of fixation. By this ap-
approach, a uniform reference point was obtained for comparisons of anatomic parameters between infarcted and noninfarcted ventricles.

**Ventricular Size and Shape**

The major intracavitary axis of the left ventricle from apex to base was measured, and two adjacent sections, midway between the base and the apex and perpendicular to the longitudinal axis of the ventricle, were obtained to measure chamber luminal diameter and the thickness of the free wall of the left ventricle and interventricular septum.18 For the measurement of chamber diameter, the minimal and maximal diameters of the ventricular chamber were determined, and their geometric mean was computed. The longitudinal axis and the transverse diameter were then used to compute chamber volume.18

Five equally spaced measurements of the left ventricular free wall and septum were collected from each slice, and their values were averaged. These determinations were done with a dissecting microscope with an ocular micrometer accurate to 0.03 mm. One month after coronary occlusion, the infarcted myocardium was grossly visible; thus, wall thickness estimations could be performed in the nonaffected portion of the free wall of the left ventricle, avoiding the region immediately bordering on the infarct as previously described.19 In the control group, comparable sites of the free wall were sampled for analysis. Infarcts were all found to be transmural and to involve different fractions of the wall. Since the interventricular septum was consistently spared, this region was evaluated in its entirety.

**Tissue Sampling**

In all rats, the whole left ventricle was serially sliced into 1–2-mm-thick rings perpendicular to the axis of the heart from the apex to the base. All but the two midsections of the individually numbered slices of the left ventricles were flat-embedded in glycol methacrylate so that the basal side was exposed for sectioning. These samples were used for the histometric determination of infarct size (see below). The middle two slices were used for sampling the region bordering the infarct and the remote myocardium for subsequent comparisons of myocyte size and shape, capillary volume fraction, capillary numerical density and diameter, diffusion distance for oxygen, and number of myocytes and capillaries across the wall, which involved light and electron microscopic determinations of araldite-embedded tissue blocks (see below).

The lack of anatomic criteria for the separation between the border zone and the remote myocardium after infarction makes sampling of these two regions difficult and arbitrary. In the present study, the border zone was considered to represent 10–20% of the tissue adjacent to the fibrotic myocardium. This area was defined by projecting the image of each slice on a digitizing tablet connected to a microprocessor computer system (final magnification, ×25).

Fifteen 1-mm-thick tissue blocks were then obtained from this region for light and electron microscopic morphometric analysis. Because of the irregularity of the boundaries of infarcts, sampling of the border zone was restricted to areas of myocardium that appeared free of bundles of connective tissue.

Because of differences of infarct size in the 28 hearts with occluded left coronary arteries, the remote myocardium was considered to be represented by the interventricular septum. This portion of the ventricle was never involved by the process of scarring, and 15 tissue blocks were collected from this zone as well. In the sham-operated group, the mid-zones of the left ventricle and septum were sampled in a similar manner. The specimens for electron microscopy were postfixed in OsO4 dehydrated in acetone, and infiltrated and embedded in araldite.

**Determination of the Volume of Viable Myocardium and Scared Tissue**

Sections from each methacrylate-embedded serial slice were cut at a thickness of 2.0–2.5 μm with a glass knife, 38 mm in length (LKB 2078 Histo Knife Maker, Pharmacia LKB Biotechnology, Piscataway, N.J.), and the Sorvall JB-4 microtome (Dupont Co., Newtown, Conn.) and mounted on glass slides.6 These sections representing six or seven uniformly spaced parallel planes through the whole left ventricle were stained with hematoxylin and eosin and used for the measurements of the fractional areas of the left ventricular free wall and interventricular septum. In the infarcted ventricles, the fractions of healthy and scarred myocardium within the free wall of the left ventricle were also measured. These values were obtained from projected images of each section (final magnification, ×25) on a digitizing tablet connected to a microprocessor computer system. The actual volumes of infarct and residual myocardium of the free wall and septum were derived by multiplying the total ventricular volume by their respective fractional volumes. The volume of the left ventricular free wall and septum in sham-operated control rats was similarly evaluated.

**Determination of Myocyte Nuclear Density and Mean Myocyte Cell Volume per Nucleus**

Sixteen plastic-embedded tissue blocks, eight from the free wall of the left ventricle and eight from the interventricular septum of each heart, were sectioned at a thickness of 0.75 μm using a microtome (model MT-1, Porter-Blum, Newtown, Conn.) and stained with methylene blue and safranin. Morphometric sampling at a magnification of ×630 consisted of counting the total number of myocyte nuclear profiles, N(n), per unit area of myocardium, A, of tissue sections in which 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 (model 105844, Wild Heerbrugg Instruments, Inc., Farmingdale, N.Y.), and a total of 30 such fields was evaluated in each of the two sampled regions. These measurements yielded the
mean number of nuclear profiles per unit area of myocardium, \( N(n)_A \), of the ventricular wall and septum, respectively.

Average nuclear length, \( D(n) \), in the wall and septum was determined by measuring 50 nuclei in each zone. These measurements were made at a magnification of \( \times 1,250 \) in longitudinally oriented myocytes viewed with a microscope having an ocular micrometer accurate to 0.5 \( \mu m \). Five blocks were cut from each region of the ventricle from each rat, with myofibers sectioned perpendicular to their length to avoid longitudinal compression. Sections approximately 2 \( \mu m \) in thickness were collected and stained, and 10 measurements of nuclear length were recorded from each tissue section. Only those nuclei in which the nuclear envelope was sharply defined at both ends and in which clusters of mitochondria were clearly visible in the areas adjacent to the nuclear edges were measured.7

Measurements of the number of myocyte nuclei per unit volume of myocardium, \( N(n)_V \), in the free wall and septum were obtained using the equation:

\[
N(n)_V = N(n)_A / D(n)
\]

The total number of nuclei, \( N(n)_T \), in the ventricular wall and septum was then computed according to the equation:

\[
N(n)_T = N(n)_V \cdot V_T
\]

where \( N(n)_V \) corresponds to the number of nuclei per unit volume of myocardium and \( V_T \) represents the total volumes of myocardium in the spared region of the free wall and septum. It follows that the aggregate volume of myocardium, \( V_T \), multiplied by the volume fraction of myocytes in the tissue, \( V(m)_V \), and divided by the total number of myocyte nuclei, \( N(n)_T \), yields the average myocyte cell volume per nucleus, \( V(m)_n \), in each of these two regions:

\[
V(m)_n = V_T \cdot V(m)_V / N(n)_T
\]

**Electron Microscopic Morphometry**

In each rat, eight blocks with myofibers oriented in the transverse direction, four from the ventricular wall and four from the septum, were thin-sectioned for electron microscopy. An additional two blocks from each region with longitudinally oriented cells were similarly prepared. Low-power electron micrographs of transverse sections of myocardium, seven from each tissue block, were collected and printed at \( \times 5,000 \). These micrographs were analyzed morphometrically with a superimposed grid consisting of 140 sampling points and 14 test-line segments each 150 mm long.20

The volume fraction of myocardial components was measured in 2,632 of these low-power micrographs, 1,064 from sham-operated controls and 1,568 from infarcted rats, by counting the fraction of sampling points overlying myocytes, capillaries, and the remaining portion of the interstitium. The numbers of myocyte and capillary profiles in the sampled area were counted to estimate their numerical densities and average cross-sectional areas and diameters. The average diffusion distance for oxygen was calculated from the capillary profile density and capillary cross-sectional area.5,20

Deviations from ideal orientation result in an underestimation of the number of structural profiles in myocardial sections. The effects of obliquity in cardiac muscle have been discussed recently with respect to numerical densities of structures and surface area measurements.21 It was concluded that to minimize the potential errors associated with oblique orientation of anisotropic structures, certain criteria have to be followed. In particular, micrographs of transversely sectioned myofibers can be used for quantitative analysis of these oriented cells only when the spacing between \( Z \) bands is at least greater than twice the true sarcomere length, measured in longitudinally oriented sections of myocytes. This approach was used in the present study.

The mean center-to-center distance, \( d_{cc} \), between myocytes was calculated from the number of profiles counted per unit area of tissue, \( N(m)A \), in transverse myocardial sections by assuming the tendency for these roughly cylindrical cells to pack in a close hexagonal pattern22:

\[
d_{cc} = \sqrt{\frac{2}{3 \cdot N(m)_A}} = 1.0746 \frac{1}{\sqrt{N(m)_A}}
\]

The preference for a hexagonal pattern in the distribution of myocytes in the myocardium was based on the morphometric measurements of cell cross section and myocyte surface/volume ratio.22 The same concept was used to estimate the average number of myocytes across the ventricular wall, that is, the number of myocytes that would be traversed by a thin transmural probe inserted perpendicular to the surface of the wall. In a hexagonal pattern, the spacing between planes of adjacent cells varies with the orientation of the hexagonal array from a maximum of \( d_{cc} \) to a minimum of \( d_{cc} \sqrt{3}/2 \) and has a mean value, \( \bar{d} \), given by

\[
\bar{d} = \frac{3d_{cc}}{\pi} \sqrt{\frac{3}{\pi}} \int_{0}^{\pi/6} \frac{d \phi}{\cos \phi} = 0.9085 d_{cc}
\]

Thus, the transmural number, \( N(m)_m \), of myocytes across a wall of thickness, \( W \), can be found from

\[
N(m)_m = W/\bar{d} = 1.0243 W \sqrt{N(m)_A}
\]

Five random fields, from longitudinally oriented sections of myocytes, were collected from each of two tissue blocks from the left ventricular wall and septum of each rat and printed at \( \times 20,000 \). Mean sarcomere length in myofibrils was obtained from 100 measurements in each region in each rat, using
sections that had been cut perpendicular to the fiber axis to avoid compression artifact. The theoretical aspects and practical applications to the morphometric procedure briefly summarized above have recently been described in detail.  

**Computation of Diastolic Wall Stress**

Diastolic wall stress, \( \sigma_W \), midway between apex and base, was computed using the Laplace equation \( \sigma_W = (p \cdot r)/2h \), where \( h \) is wall thickness, \( 2r \) is chamber diameter obtained at death, and \( p \) is the in vivo estimation of left ventricular end-diastolic pressure. In a similar manner, diastolic septal stress was calculated. These calculations assumed a circular transverse shape of the ventricle. In this regard, myocardial infarction does not alter this configuration, and small variations were taken into account by averaging maximum and minimum diameters at the equator through a geometric mean calculation, as described above.

The knowledge of ventricular pressure, chamber diameter, and wall thickness in diastole allows a further analysis of diastolic stress in terms of its distribution across the ventricular wall. This parameter can be derived from the equation elaborated by Mirsky:  

\[
\sigma_W = p \cdot \frac{a^2 + b^2/2(R)^3}{b^3 - a^3}
\]  

where \( p \) corresponds to ventricular pressure, \( R \) is the radial coordinate, and \( a \) and \( b \) are the inner and outer radii, respectively. It should be pointed out, however, that \( R \) can be substituted by the number of cells across the wall, \( N(m)_{wa} \), which describes increments in wall thickness from the endocardium to the epicardium. Thus, Equation 7 was integrated with this information to yield stress distribution (\( \sigma \)) at the cellular level of organization:

\[
\sigma_c = p \cdot \frac{a^2 + b^2/2([W/N(m)_{wa}]C_b)^3}{b^3 - a^3}
\]  

where \( W_t \) is the ventricular wall thickness, \( N(m)_{wa} \) is the number of cells across the wall, and \( C_b \) is the cell layer to be examined with the endocardial surface = 0 and the epicardial surface = \( N(m)_{wa} \). It should be noted that these determinations were restricted to the regions of the wall at the equator occupied by viable myocardium since the inhomogeneity of structures and the lack of a structural reference point in the injured areas would complicate the computation of regional stress values.

**Sampling Size**

The magnitude of sampling used in this investigation was selected on the basis of previous work performed in our laboratory and the principle of Poisson statistics. The latter can be used as a reasonable guideline for morphometric data collection, since it provides a somewhat more conservative estimate of necessary counts than more specific formulations derived for point counts and profile counts. By assuming that biological variability among animals in a given experimental group is approximately 10%, counting errors in each animal should also be limited by the same order of magnitude for the least frequent structure. In the present study, the area of myocardium sampled by light microscopy yielded an average of 454 and 482 nuclear profiles counted in the free wall and interventricular septum, respectively, of each sham-operated control rat. Values of 332 and 410 were obtained in each region of the ventricle of each rat with small infarcts. In rats with large infarcts, counts of 262 and 388 were collected. Corresponding sampling errors of these measurements are 4.7% and 4.6% in controls, 5.6% and 4.9% in rats with small infarcts, and 6.2% and 5.1% in rats with large infarcts.

The area of myocardium examined by electron microscopy resulted in the sampling of 154 and 161 myocyte profiles in the wall and septum of each control rat. The numbers of capillary profiles in these regions were 133 and 137. These values yielded sampling errors of 8.1% and 7.9% for myocytes and 8.7% and 8.5% for capillaries. In the rats with small infarcts, 134 and 152 myocytes were sampled in the wall and septum of each rat. Capillary values were 106 and 127. Corresponding sampling errors were 8.6% and 8.1% for myocytes and 9.7% and 8.1% for capillaries. After large infarcts, myocyte counts in the wall and septum were 113 and 148, and capillary counts were 93 and 126. Thus, sampling errors were 9.4% and 8.2% for myocytes and 10.4% and 8.9% for capillaries.

The nested analysis of variance performed after the code was broken demonstrated that the number of blocks sampled, the number of micrographs collected from each block, and the number of sampling points and profile counts were in excess of what would have been the minimum required for optimum efficiency.

**Data Collection and Analysis**

All tissue samples were coded, and the code was broken at the end of the experiment. Results are presented as mean±SD computed from the average measurements obtained from each rat. Statistical significance in multiple comparisons in which the analysis of variance and the \( F \) test indicated the presence of significant differences was determined by the Bonferroni method. Values of \( p<0.05 \) were considered significant.

**Results**

Results pertaining to the determination of infarct size are presented first, to subsequently express functional data in relation to the magnitude of ventricular damage produced by coronary artery ligation.
Determination of Infarct Size

The morphometric analysis of the serial sections of the infarcted hearts demonstrated that the fibrotic area occupied a variable portion of the myocardium of the free wall of the left ventricle. The 28 infarcted hearts were then subdivided into two groups: those with small ventricular infarcts and those with large infarcts. The first group of 15 ventricles included infarcts comprising up to 12% of the wall (scar, 7.48 \( \pm \) 3.97%; viable myocardium, 92.52 \( \pm \) 3.97%); the second group of 13 ventricles included infarcts involving more than 12% of the wall (scar, 17.30 \( \pm \) 2.38%; viable myocardium, 82.70 \( \pm \) 2.38%). This initial analysis was subsequently expanded by measuring the percent loss of myocyte nuclei in the ventricular myocardium as previously described.\(^4,6,7,12\) This quantity provides an estimation of infarct size that is independent of the tissue and cellular processes accompanying the remodeling of the ventricle with time.\(^4,7,12\)

Figure 1 shows the data concerning the numerical density of myocyte nuclei per cubic millimeter of myocardium (panel A), which, in combination with the total volume of viable myocardium (panel B), allows the calculation of the total number of myocyte nuclei present in the free wall of the left ventricle of each rat group (panel C). The 30% reduction (\( p<0.0001 \)) in myocyte nuclei numerical density, in association with an 11% decrease (\( p<0.005 \)) in free wall myocardial mass, resulted in a 38% lower (\( p<0.0001 \)) total number of myocyte nuclei in rats with small infarcts. An identical analysis of large infarcts demonstrated that the 45% (\( p<0.0001 \)) and 29% (\( p<0.0001 \)) diminutions in nuclear numerical density and myocardial volume provoked a 60% decrease (\( p<0.0001 \)) in the aggregate number of myocyte nuclei in the ventricle.

Gross Cardiac Characteristics

After infarct size was defined both histometrically and morphometrically, the gross cardiac characteristics of the rats with 38% (small) and 60% (large) infarct averages were analyzed and compared. The occurrence of myocardial infarction was found to be associated with a reduced body weight gain (Figure 2A), which was similar in the two infarcted rat groups. Although no difference in total heart weight (Figure 2B) was seen between sham-operated controls and rats with small infarcts, a 6% increase (\( p<0.01 \)) in this parameter was noted in rats with large infarcts. Furthermore, the weight of the left ventricle inclusive of the septum remained essentially constant 30 days after coronary artery occlusion (Figure 2C). In contrast, in comparison with sham-operated controls and rats with a 38% infarct, the right ventricle in rats with large infarcts hypertrophied by 29% (\( p<0.0001 \)) and 22% (\( p<0.0001 \)), respectively (Figure 2D).

Functional Determinations

Physiological measurements taken just before death showed that mean arterial blood pressure was not affected by a 38% infarct, whereas a 60% loss of viable myocardium resulted in a 23% decrease (\( p<0.0001 \)) in this parameter. In addition, a 19% difference (\( p<0.001 \)) in mean arterial pressure was measured between the two infarct groups (Figure 3A). As illustrated in Figure 3B, large infarcts also produced a 20% reduction (\( p<0.0001 \)) in left ventricular systolic pressure, which, however, remained unchanged in rats with small infarcts. Although mean arterial pressure and peak systolic ventricular pressure were not altered by a 38% infarct, left ventricular end-diastolic pressure was found to be increased from 2 to 6 mm Hg (Figure 3C). In the presence of a 60% infarct, left ventricular end-diastolic pressure reached a value of 16 mm Hg, which was markedly higher than that measured in control rats (\( p<0.0001 \)) and in rats with small infarcts (\( p<0.0001 \)). In addition, estimations of dP/dt demonstrated an 8% (\( p<0.05 \)) and a 31% (\( p<0.0001 \)) reduction in rats
with small and large infarcts, respectively (Figure 3D). Large infarcts also showed a 25% (p<0.0001) lower value for dP/dt than did small infarcts.

Ventricular Remodeling and Wall Stress

Figure 4 depicts the changes in transverse chamber diameter and in the thickness of the free wall and septum as a consequence of myocardial infarction. It was stated in “Materials and Methods” that, because of the variability in infarct size and the lack of an anatomic landmark separating the surviving myocardium bordering the infarct from that away from the fibrotic tissue, the interventricular septum was assumed to be representative of the myocardium remote from the area of ischemic damage in all cases. On the other hand, the border zone comprised 10–20% of the spared myocardium near the fibrotic tissue, as previously described. Therefore, the parameters illustrated in Figure 4 as free wall and septum are intended to characterize the properties of the border zone and remote myocardium, respectively.

One month after coronary artery occlusion, transverse chamber diameter increased by 6% (p<0.05) and 27% (p<0.0001) in rats with small and large infarcts, respectively. Moreover, large infarcts provoked a 20% (p<0.0001) greater ventricular dilation than did small infarcts (Figure 4A). Since the thickness of the ventricular wall bordering and remote from the infarct increased by 8% (p<0.05) in rats with small infarcts and remained essentially constant in rats with large infarcts (Figure 4B), the wall thickness/chamber radius ratios in the border zone

Figure 2. Bar graphs showing effects of coronary artery occlusion on body weight and gross cardiac characteristics. SO, sham-operated rats; SI, rats with small infarcts; LI, rats with large infarcts. *Significantly different at p<0.05 compared with sham-operated control rats. **Significantly different at p<0.05 compared with rats with small infarcts.

Figure 3. Bar graphs showing effects of coronary artery occlusion on mean arterial pressure, left ventricular systolic pressure, left ventricular end-diastolic pressure, and left ventricular dP/dt. SO, sham-operated rats; SI, rats with small infarcts; LI, rats with large infarcts. *Significantly different at p<0.05 compared with sham-operated control rats. **Significantly different at p<0.05 compared with rats with small infarcts.
and remote myocardium did not vary in rats with small infarcts but markedly decreased in rats with a 60% infarct (Figure 4C).

To establish whether the dimensional changes of the infarcted ventricle in combination with the impairment of cardiac hemodynamics resulted in alteration in tension on the surviving myocardium, diastolic stress was computed from Laplace's equation (Figure 4D). This parameter increased by 2.4-fold \((p<0.005)\) and 2.3-fold \((p<0.02)\) in the border and remote regions in rats with small infarcts. In the presence of large infarcts, augmentations of 8.8-fold \((p<0.0001)\) and 9.3-fold \((p<0.0001)\) were measured in the zones adjacent and away from the scarred tissue. Moreover, in rats with large infarcts, diastolic stress was 3.7-fold \((p<0.0001)\) and 4.0-fold \((p<0.0001)\) greater than diastolic stress in rats with small infarcts in the border and remote tissues, respectively.

Additional characteristics pertinent to the recognition of the magnitude of ventricular remodeling associated with infarction are illustrated in Figure 5. The major longitudinal axis of the ventricle increased by 11% \((p<0.01)\) in rats with large infarcts (Figure 5A). Moreover, the computation of chamber volume showed that marked augmentations in ventricular cavity size occurred with infarction. In rats with small infarcts, a 15% expansion in chamber volume was found (Figure 5B), but this change was not statistically significant. On the other hand, large infarcts were accompanied by an 81% increase in cavity volume.
volume; this chamber enlargement exceeded by 57% the dilation generated by small infarcts (Figure 5B).
In view of the fact that the volume of ventricular mass constituted by the surviving myocardium of the left ventricular free wall and septum combined decreased by 10% ($p<0.0005$) in rats with large infarcts (Figure 5C), a 50% reduction in ventricular mass/chamber volume ratio was measured in this case (Figure 5D).
In rats with small infarcts, the 15% decrease in ventricular mass/chamber volume ratio was also found to be statistically significant (Figure 5D).

**Cellular Adaptations**

Average myocyte cell volume per nucleus was determined in the border and remote portions of the surviving myocardium in each of the two infarcted rat groups and in corresponding regions of sham-operated control rats. Figure 6 shows the dimensional characteristics of left ventricular myocytes after infarctions involving a 38% and a 60% average of the free wall of the left ventricle 1 month after coronary artery occlusion. By comparing the values obtained in rats with small infarcts with those measured in controls, it was seen that cell size increased by 43% ($p<0.0001$) in the border zone and by 20% ($p<0.0001$) in the zone away from the scarred tissue (Figure 6A). After large infarcts, the cellular responses were 81% ($p<0.0001$) and 32% ($p<0.0001$). Moreover, mean cell volume per nucleus in the group of rats with large infarcts was 26% ($p<0.0001$) and 10% ($p<0.02$) greater than that found in the border and remote regions of rats with small infarcts.

Figure 6 illustrates the changes in myocyte diameter (panel B) and length (panel C) associated with these cellular enlargements. In the presence of small infarcts, myocyte hypertrophy per nucleus in the myocardium adjacent to the scar was produced by a 7% ($p<0.01$) increase in the average diameter and a 25% ($p<0.005$) augmentation in mean length. Corresponding increases in the presence of large infarcts were 17% ($p<0.0001$) and 33% ($p<0.0001$). Changes of smaller magnitude were noted in the remote myocardium. Cell lengthening per nucleus averaged 13% ($p=NS$) and 19% ($p<0.005$) in the presence of small and large infarcts (panel C). The lateral expansion of myocytes was 3% ($p=NS$) and 5% ($p<0.05$). Finally, as a result of a 60% infarct, the diameter of myocytes in the border zone was 9% ($p<0.001$) greater than that of the corresponding cells adjacent to infarcts comprising 38% of the ventricular wall (panel B).

These changes in myocyte shape all occurred in the presence of constant sarcomere lengths. Sarcomere length in the free wall was $2.01 \pm 0.10$, $2.03 \pm 0.09$, and $2.06 \pm 0.12 \mu m$ in controls, rats with small infarcts, and rats with large infarcts, respectively. Corresponding values in the septum were $2.04 \pm 0.13$, $2.06 \pm 0.11$, and $2.05 \pm 0.11 \mu m$.

**Capillary Adaptations**

The volume fraction of capillaries in the myocardium, the transverse diameter of capillary profiles, and the numerical density of capillaries in the region bordering on the infarct and remote from the scarred tissue are shown in Figure 7. The preservation of the volume percent of capillaries within the surviving myocardium of the border zone (panel A) in rats with small and large infarcts was due to an increase in capillary diameter (panel B) that compensated for the reduction in capillary number per square millimeter of tissue (panel C). Capillary diameter increased by 14% ($p<0.0001$) in the border zone in rats with small and large infarcts. Capillary numerical density, however, was reduced by 21% ($p<0.0001$) in the former and by 30% ($p<0.0001$) in the latter. No statistically significant differences in these capillary properties were noted in the remote myocardium for both infarct sizes.

Figure 7D shows the changes in the diffusion distance for oxygen associated with myocardial infarction. This parameter was found to be consistently

**Figure 6.** Bar graphs showing effects of coronary artery occlusion on myocyte cell volume and length per nucleus in the surviving tissue. FW, free wall; S, interventricular septum; SO, sham-operated rats; SI, rats with small infarcts; LI, rats with large infarcts. *Significantly different at $p<0.05$ compared with sham-operated control rats. **Significantly different at $p<0.05$ compared with rats with small infarcts.
increased 1 month after coronary artery occlusion. The major increase was measured in the border zone in rats with large infarcts: a value 23% (p<0.0001) and 10% (p<0.01) greater than that observed in the corresponding region of sham-operated controls and rats with small infarcts was noted. When the ratios of capillary/myocyte profiles were computed, it was demonstrated that no alterations occurred in these relations in all cases (data not shown).

Architectural Rearrangements of the Wall

As indicated in “Materials and Methods,” the availability of the numerical density of myocytes and capillaries per square millimeter of myocardium and wall thickness allows the quantitative estimation of the numbers of myocytes and capillaries that would be encountered by a probe perpendicular to the ventricular surface traversing the entire width of the wall.22 Figure 8 illustrates that 1 month after an infarction resulting in a 60% loss of mass there was a 12% reduction (p<0.005) in the average number of myocytes across the region of the wall bordering the fibrotic tissue, in spite of the fact that wall thickness in this zone of the injured ventricle was similar to that of control hearts (Figure 4B). Moreover, the capillary number within the thickness of the wall was also decreased by 14% (p<0.0001). In the remote myocardium, both myocytes and capillaries were reduced by 8% (p<0.025). No change occurred in these myocyte and capillary quantities in rats with a 38% infarct.

By use of the calculations of the number of myocytes across the wall and Equation 8, the distribution of diastolic stress at the cellular level was deduced (Figure 9). Myocardial infarction produced a marked elevation in diastolic cell stress that involved mostly the inner two thirds of myocytes of the border and remote regions of the injured ventricle. The augmentation in cell stress was greater in rats with large infarcts. This was due, in part, to the mechanism of side-to-side slippage of cells within the wall and

**Figure 7. Bar graphs showing effects of coronary artery occlusion on the capillary characteristics in the remaining myocardium. FW, free wall; S, interventricular septum; SO, sham-operated rats; SI, rats with small infarcts; LI, rats with large infarcts. *Significantly different at p<0.05 compared with sham-operated control rats. **Significantly different at p<0.05 compared with rats with small infarcts.**
Infarction and levels altered was wall and different from controls. operated wall and controls. Moreover, diastolic overloading appears to affect mostly the inner two thirds of the spared myocytes within the wall acutely and chronically; chronic overloading was seen in the present study 1 month after the imposition of myocardial infarction. It should be pointed out that the calculations of diastolic stress in this investigation did not take into account the changes in the volume composition of the spared myocardium after infarction. Inhomogeneity and differences between tissues may affect wall and cell stress values. However, the hypertrophic reaction of the viable myocardium did not significantly alter the relative proportion of its major structural constituents. For example, the volume fraction of myocytes was found to be 82.92±2.10%, 83.42±1.82%, and 85.00±1.99% in the septic of the control, small-infarct, and large-infarct groups, respectively. Corresponding values in the free wall were 82.76±1.50%, 82.53±1.05%, and 83.03±3.43%.

The computation of systolic wall stress after infarction is complicated by the fact that it is difficult to predict how the shape of the injured ventricle changes during systolic contraction. However, by assuming that systole affects fiber length by 15% in control animals, systolic wall and septal stresses were calculated from diastolic measurements and were found to be 840 dyne/mm² and 1,107 dyne/mm², respectively. Even under a very unlikely condition of little or no shortening in the infarcted ventricle, systolic stress in the border and remote regions in the presence of a 38% infarct would be 1,010 dyne/mm² and 1,317 dyne/mm². Corresponding values for a 60% infarct would be 1,134 dyne/mm² and 1,604 dyne/mm². Thus, at most, systolic stress would increase by nearly 20% in the perinfarcted and remote myocardium with small infarcts and by 35% and 45% in the tissue adjacent and away from the scar in the presence of large infarcts. Since these derivations are overestimations of the actual magnitude of systolic stress, diastolic overloading appears to constitute the prevailing hemodynamic abnormality in healed myocardial infarction. A similar condition has also been found acutely in the same animal model. On such a basis, it is tempting to speculate that severe ischemic injury of the myocardium leads to diastolic dysfunction that is not reversed by the hypertrophic

FIGURE 9. Graphs showing effects of coronary artery occlusion on the distribution of diastolic wall stress at the cellular level within the surviving myocardium of the left ventricle. Each individual value in rats with small and large infarcts was significantly different from the corresponding value in sham-operated controls. △, Large infarcts; ○, small infarcts; ◻, controls.

Discussion

The findings of the current study indicate that the chronic hypertrophic response of the surviving myocardium after infarction is not capable of normalizing ventricular pump performance and diastolic wall and myocyte cell stress when the ischemic event leads to a loss of myocytes of nearly 40% or more of the left ventricular free wall. Although reactive growth processes in myocytes result in a substantial recovery of tissue mass, dilation of the ventricular chamber, side-to-side slippage of myocytes and capillaries within the wall, and inadequate mural thickening all contribute to maintain elevated levels of myocardial and cellular loads that may sustain the progression of the disease state toward end-stage heart failure and death.

Diastolic and Systolic Laplace Loading and Myocardial Infarction

The current results demonstrate that 1 month after coronary artery ligation, ventricular pump function was altered in infarcts affecting 38% and 60% of the free wall of the left ventricle. The depressed cardiac performance was documented by increases in left ventricular end-diastolic pressure and reductions in dP/dt. The magnitude of impairment in ventricular hemodynamics was greater in the large-infarct group, in which mean arterial blood pressure and peak systolic ventricular pressure were also decreased. In both infarct groups, the changes in ventricular pressure in association with the increases in chamber diameter resulted in a marked elevation in diastolic wall stress. This parameter augmented 2.4-fold in small infarcts and 9.0-fold in large infarcts. A similar alteration in diastolic wall stress has been found 2 days after coronary artery occlusion in infarcts comprising a 63% average of the left ventricular wall. Moreover, diastolic overloading appears to affect mostly the inner two thirds of the spared myocytes within the wall acutely and chronically; chronic overloading was seen in the present study 1 month after the imposition of myocardial infarction. It should be pointed out that the calculations of diastolic stress in this investigation did not take into account the changes in the volume composition of the spared myocardium after infarction. Inhomogeneity and differences between tissues may affect wall and cell stress values. However, the hypertrophic reaction of the viable myocardium did not significantly alter the relative proportion of its major structural constituents. For example, the volume fraction of myocytes was found to be 82.92±2.10%, 83.42±1.82%, and 85.00±1.99% in the septic of the control, small-infarct, and large-infarct groups, respectively. Corresponding values in the free wall were 82.76±1.50%, 82.53±1.05%, and 83.03±3.43%.
reactive process of surviving muscle fibers. Moreover, a diastolic Laplace overload is consistent not only with the gross anatomic characteristics of the injured ventricle but also with the predominant increase in myocyte length measured at different stages of healing after infarction.\textsuperscript{4,7} It should be pointed out, however, that the method of fixation used in the present study most likely resulted in an underestimation of chamber luminal diameter and an overestimation in the thickness of the wall. These effects may have provoked an underestimation of both systolic and diastolic wall stress calculations. Such a limitation has to be considered in the assessment of the actual values of wall stress claimed in the present study and in their implication in wall remodeling after infarction.

**Cellular Basis of Infarction-Induced Decompensated Eccentric Hypertrophy**

The present results indicate that cellular growth mechanisms after infarction paralleled not only the extent of myocardium lost on an obligatory basis but also the magnitude of ventricular dilatation measured 1 month after coronary artery occlusion. Moreover, myocyte enlargement differed in the region bordering the scarred tissue and in the zone away from the scar. In rats with small infarcts, the average myocyte cell volume per nucleus increased by 43\% and 20\% in the border and remote myocardium, respectively. In both cases, the expansion in length of the cells, 25\% and 13\%, exceeded the augmentation in the lateral dimension, 7\% and 3\%. In rats with large infarcts, myocyte size in the border zone enlarged by 81\% through a 33\% increase in length and a 17\% augmentation in transverse diameter. Cell volume per nucleus far from the fibrotic myocardium hypertrophied by 32\%, through a 19\% lengthening and a 5\% expansion in cross-sectional diameter. Thus, the magnitude of hypertrophy of the spared myocytes in the region bordering the scarred tissue was greater than that in the portion remote from the infarct, and this difference increased with infarct size.

Several studies in recent years\textsuperscript{4,6,7} have shown that myocyte cellular hypertrophy occurs after myocardial infarction. Myocyte enlargement is an early event\textsuperscript{4} that progresses throughout the healing process.\textsuperscript{6,7} Moreover, infarcts comprising a large portion of the free wall of the left ventricle lead to hypertrophy of the left atrium with enlargement of both mononucleated and binucleated myocytes.\textsuperscript{27} When left-side pump failure is present,\textsuperscript{4,8,10} the right ventricle hypertrophies\textsuperscript{8,10} by enlarging its myocyte population\textsuperscript{4,28} in an attempt to maintain the pressure gradient across the pulmonary bed.\textsuperscript{4,10} Such a response of the right side of the heart was confirmed in the present study: in rats with large infarcts, a 29\% augmentation in right ventricular weight was measured. Furthermore, in the presence of extensive infarcts, the addition of contractile mass in the injured ventricle by muscle cell hypertrophy has been shown to be inadequate for a complete reconstitution of the myocyte compartment of the myocardium.\textsuperscript{7} Thus, cardiac myocytes appear to be unable to offset by cellular hypertrophy alone the loss of mass produced by infarcts involving 50\% or more of the ventricle. It remains to be determined, however, whether longer time periods after the acute ischemic event, in combination with a prolonged stress on the surviving cells, may trigger a hyperplastic reaction in myocytes with further recovery of functioning tissue. This possibility seems to be operative in a variety of pathological states that impose a large and sustained overload on the myocardium of both humans\textsuperscript{29} and animal models.\textsuperscript{22,30}

The hypertrophic growth of the myocyte population after infarction was characterized by increases in myocyte diameter and length, which both contribute to cellular enlargement in the presence of small and large infarcts of the left ventricle. These cellular shape changes have been shown to be consistent with a phenomenon of concentric and eccentric hypertrophy in the intact ventricular wall\textsuperscript{31-33}; the lateral expansion of myocytes leads to a corresponding increase in wall thickness,\textsuperscript{31} whereas myocyte lengthening is associated with a proportional augmentation in chamber volume.\textsuperscript{31} In the presence of large infarcts, an additional structural mechanism was implicated in ventricular dilatation and relative wall thinning. The number of myocytes across the wall in the peri-infarcted region was decreased by 12\%, whereas the mural number of capillaries was reduced by 14\%. Smaller reductions in myocyte and capillary numbers were seen in the remote myocardium. Such a phenomenon of side-to-side slippage of cells and capillaries within the wall, which is acute after coronary occlusion,\textsuperscript{12} has been demonstrated to be an important factor in the dilatation of the infarcted ventricle. However, in spite of the fact that infarct size was approximately equal to that measured here, the magnitude of the process was significantly greater,\textsuperscript{12} raising the possibility of the reversibility of this event. Thus, negative side-to-side slippage of cells with wall thinning early after ischemic injury may be counteracted later during healing by an opposite positive movement of cells and wall thickening. The present observations, in combination with previous findings,\textsuperscript{12} appear also to suggest that the phenomenon of muscle fiber slippage is a function of infarct size and that it affects mostly the border zone of an infarct both acutely and chronically.

**Myocardial Infarction and Ventricular Dimensions**

The findings of the present study demonstrate that a segmental loss of ventricular mass resulted, over a period of 1 month, in an increase in ventricular chamber volume. Infarcts affecting an average 38\% of the wall of the left ventricle were characterized by a 15\% augmentation in chamber volume, mostly due to a 6\% expansion of the transverse cavity diameter. On the other hand, infarcts with a 60\% loss of contractile mass were associated with an 81\% enlargement in chamber volume, brought about through a 27\% and an 11\% increase in the transverse...
and longitudinal axes of the ventricular cavity, respectively. The changes in volume of the ventricular chamber were greater than those involving the growth response of the viable myocardium. Mass/volume ratio was decreased by 15% in rats with small infarcts and by 50% in rats with large infarcts.

The anatomic alterations of the infarcted ventricle summarized above are consistent with ventricular shape changes characteristic of decompensated eccentric hypertrophy, the form of hypertrophy that distinguishes the transition from compensated pressure and/or volume overload hypertrophy to myocardial dysfunction and failure. An increasing pressure load induces concentric ventricular hypertrophy in which wall thickness increases without chamber enlargement, leading to an augmentation in the mass/volume ratio. On the other hand, an increased volume load typically induces enlargement of the ventricular chamber volume and a corresponding expansion in myocardial tissue volume in order to retain a constant mass/chamber volume ratio. These factors characterize eccentric hypertrophy in the well-balanced stage, in which chamber dilation is also accompanied by a proportional increase in wall thickness, so that the wall thickness/chamber radius ratio is not altered. When these relations are not preserved, decompensated eccentric hypertrophy develops, as shown here after myocardial infarction. In the presence of a 38% infarct, however, some degree of compensation at the anatomic level was apparent, since the wall thickness/chamber radius ratio was not decreased. In the presence of a 60% infarct, this ratio was reduced by 19%.

Ventricular dilation and wall thinning has previously been shown acutely and chronically after myocardial infarction. Moreover, this unfavorable evolution has been demonstrated not to be restricted to large infarcts only. Pfeffer and coworkers have shown that the administration of an angiotensin converting enzyme inhibitor can reduce the magnitude of ventricular remodeling and chamber dilation. Similar observations have recently been made in humans as well. However, it remains to be determined whether the angiotensin converting enzyme inhibitors alter favorably and chronically the cardiovascular adaptation of the infarcted heart. The mechanism of this protective effect may be due to 1) an increase in the myocardial mass/chamber volume ratio, 2) an augmentation of the wall thickness/chamber radius ratio, 3) a preservation of the transverse chamber diameter/longitudinal chamber axis ratio, or 4) a combination of these three factors.

**Heterogeneity in the Myocardial Response to Infarction**

The present findings indicate that the hypertrophic response of myocytes in the region bordering the scarred tissue was greater than that in the portion remote from the infarct and that this difference was greater with larger infarcts. Moreover, the decrease in capillary density affected the peri-infarcted region more than the tissue away from the scar. Finally, the diffusion distance for oxygen increased to a greater extent in the border zone. Thus, as previously suggested, the oxygenation potential of the surviving myocardium is significantly reduced, and such an imbalance is more apparent in the zone adjacent to the healed area where the cells may be more susceptible to additional ischemic episodes, a phenomenon commonly seen during reinfarction.

Although the border zone of a myocardial infarct is generally considered to represent tissue adjacent to the central area of maximal ischemia, the hypertrophic response in this area is acute after coronary occlusion, and the possibility is raised that such a condition may also be present chronically after the completion of the healing process. The interaction of the area of infarction with the viable myocardium may place obligatory constraints on the latter, resulting in altered series elasticity and an increased tension load, superimposed on the augmentation in load associated with increased ventricular diameter and diastolic filling pressure. Such an overload may be responsible for the magnified hypertrophic cellular reaction in this region of the wall and the consequent impairment in capillary density and diffusion distance for oxygen.

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