Cellular Mechanisms of Myocardial Infarct Expansion

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Infarct expansion is acute regional dilatation and thinning of the infarct zone. There are several possibilities for the mechanism of this alteration in cardiac shape: thinning could be caused by 1) cell rupture, 2) a reduction in the intercellular space, or 3) stretching of myocytes or 4) slippage of groups of myocytes so that less cells are distributed across the wall. To determine the relative contributions of these cellular mechanisms of wall thinning and dilatation, detailed study of transverse histological sections of rat hearts with infarct expansion was performed 1, 2, and 3 days after coronary ligation. The number of cells across the wall was determined in six regions within, adjacent to, and remote from the infarct. Cell counting was performed so that the total number of cells across the wall and the number of cells per unit length (cell density) across the wall were determined. The transmural cell count and the cell density were correlated with the wall thickness in each region. Myocyte cross-sectional areas and sarcomere lengths were also measured. The results from the infarct expansion hearts were compared with those of sham-operated control hearts that had been similarly analyzed. To ensure that mechanisms identified in the rat were applicable to human infarct expansion, five hearts from patients who died within 3 days of infarction and two hearts from patients without coronary disease were studied histologically in a similar fashion. Wall thinning occurred in all regions of the rat infarct expansion hearts compared with controls ($p < 0.0001$) but, as expected, was most pronounced in the infarct zone. A decrease in the number of cells across the wall accompanied the wall thinning at each site ($p < 0.0001$), and this change in cell number was highly correlated with the changes in wall thickness ($r = 0.915, p < 0.001$). Cell density increased from controls only within the infarct zone ($p < 0.001$) and accounted for at most 20% of the thinning in that region. The change in cell density was attributable to both cell stretch (measured by increased sarcomere length and decreased myocyte cross-sectional area) and a decrease in the intercellular space. A similar strong correlation between wall thinning and decreased number of cells across the wall was identified in the human hearts ($r = 0.94, p < 0.001$). Thus, a decrease in the number of cells across the wall accounts for most wall thinning in the infarct zone and all thinning in noninfarcted regions. In noninfarcted regions, cell slippage accounts for all thinning. Cell stretch and loss of intercellular space are confined to the infarct zone and contribute less to wall thinning than does cell slippage. (Circulation 1988;78:186–201)

Infarct expansion describes the disproportionate thinning and dilatation of acutely infarcted myocardium that occurs within the first 24 hours after infarction and leads to early cardiac dilatation. These early structural changes within the infarct zone are accompanied by similar but less pronounced changes in wall thickness and radius of curvature in other regions of the left ventricle, even those remote from the infarct. However, the precise mechanism by which the heart alters its shape acutely after infarction is unknown.

Previous studies examining the mechanism of ventricular dilatation have suggested at least five kinds of cellular changes that could explain these gross alterations in cardiac architecture. 1) Myocyte hypertrophy by addition of sarcomeres in series is

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one means of producing myocyte elongation and chamber enlargement.6–8 Of course, this type of hypertrophy could not account for thinning and dilatation within the infarct zone because the myocytes are dead, but it might explain some of the gross shape changes outside the infarct region.9 However, cellular hypertrophy is unlikely to account for all of the immediate early postinfarction dilatation of normal regions because gross structural alterations caused by cellular hypertrophy are not usually seen in the first few days after an inciting stimulus.6 2) Necrosis of myocytes also can contribute to wall thinning and dilatation.10,11 Loss of cells is unlikely to account for the acute wall thinning of infarct expansion because infarct expansion begins before resorption of necrotic myocardium. However, rupture and pulling apart of individual myocytes could explain some wall thinning. 3) Because the microvascular bed between myocytes is no longer perfused within the infarct zone, a reduction in the intercellular space (Figure 1C) might result, thereby contributing to wall thinning.12 Wall thinning by this mechanism would be limited, however, because the contribution of capillaries and other interstitial elements to overall ventricular wall volume is small relative to that of myocytes.13 4) A fourth possible mechanism of the shape change is that individual myocytes stretch (Figure 1B), resulting in cell lengthening and thinning, which would account for both the wall thinning and cavity dilatation.11,14,15 Finally, rearrangement of myocytes (Figure 1D) could occur, whereby groups or bundles of myocardial cells slip and move apart without disrupting individual cells or altering individual cell dimensions. The purpose of the present study was to distinguish the contributions of myocyte rupture, reduction in the intercellular space, cell stretch, and slippage of myocyte bundles to infarct expansion.

We used quantitative cell counting and morphometry of myocardium from rats subject to coronary ligation. Complementary results from human hearts are also reported. These studies show that slippage of myocardial cell bundles is the predominant mechanism of infarct expansion.

Subjects and Methods

We used the rat model of infarct expansion described previously2 for detailed morphologic study of the cellular mechanisms of infarct expansion. However, to ensure that mechanisms of infarct expansion found in the rat fairly represented human infarct expansion, five hearts of humans who died within 3 days of acute infarction and were found at autopsy to have infarct expansion were also studied.

Rat Infarct Model

Sixty female 225–300-g Sprague-Dawley rats were anesthetized with sodium methohexital (35 mg/kg body wt i.p.). Intermittent positive pressure ventilation was given with 95% O₂–5% CO₂. The hearts were exposed through left intercostal thoracotomy. The left coronary artery was then ligated within 2–3 mm left of the proximal aorta, and the chest was closed. The rats were subsequently maintained on standard rat chow. At 1, 2, and 3 days after infarction, the animals were given heparin intraperitoneally and anesthetized with methohexital, and the hearts were rapidly excised and submerged in cold 30 mM KCl to achieve diastolic cardiac arrest.

Histological preparation. Immediately after cardiac arrest, the hearts were fixed by intracoronary perfusion with cold 10% formalin solution for 20 minutes by cannulating the ascending aorta with polyethylene tubing connected to a gravity flow apparatus containing the cold fixative. The hearts were vented through the mitral valve with polyethylene tubing connected to a gravity flow apparatus containing the cold fixative. The hearts were then placed in 10% formaldehyde solution for 48 hours before being cut into 1-cm3 segments for histologic study.

FIGURE 1. Schematic drawings of possible mechanisms of infarct expansion. Panel A: Myocytes (stippled cylinders) and capillaries (solid cylinders) are shown as being arranged in discrete bundles (1 and 2). Panel A is a three-dimensional perspective diagram of a hypothetical preinfarct ventricular wall. In subsequent panels, only the two-dimensional cross-sectional view is shown. For illustrative purposes, cross-sections of only two bundles are shown composing the total wall thickness (t). Panel B: With cell stretch, the total number of cells across the wall and capillary dimension remain unchanged, but the number of cells per unit length (cell density) increases as the wall thins. Panel C: Collapse of the microvascular space results in tighter packing of the myocytes also producing an increase in cell density and hence wall thinning. Panel D: With rearrangement of myocyte bundles, fewer cells make up the thickness of the wall, but the number of cells per unit length is unchanged.
FIGURE 2. Drawing of rat heart cross-section showing the six regions analyzed. Region 1 is the area of maximum thinning within the infarct; Regions 2 and 6 are the anterior and posterior border zones, respectively, just inside the infarct; Regions 3 and 5 are the posterior and anterior border zones just outside of the infarct; Region 4 is in the remote noninfarcted septum.

ethylene tubing, permitting a constant coronary perfusion pressure of 60 mm Hg under zero intracavitary left ventricular pressure. After this, the hearts were sliced transversely, parallel to the atroventricular groove, in 2-mm sections from apex to base. In three control hearts and five 1-day infarct hearts, the middle 2-mm slice was divided in half transversely. Specimens for plastic embedding were obtained from the lower of the two middle 1-mm slices and fixed with 3% glutaraldehyde. The top 1-mm center slice from these hearts, as well as all other slices, continued formalin fixation by immersion for 24 hours. The formalin-fixed slices were embedded in paraffin and stained with hematoxylin and eosin and Verhoeff-Van Gieson stains.

The glutaraldehyde-fixed specimens were postfixed with osmium tetroxide, dehydrated in a graded series of alcohols and xylene, and then embedded in epoxy resin. The epoxy resin–embedded specimens were measured for cell cross-sectional area by light microscopy on semithin sections (1 μm) and for sarcomere length by electron microscopy. Selection for final processing of the epoxy resin–embedded samples was made after the paraffin sections had been processed and morphologically evaluated (see below) so that cell cross-sectional area and sarcomere-length measurements could be made within identified topographic regions. Semithin sections of the epoxy resin–embedded tissue were stained with toluidine blue and examined with a light microscope. Ultrathin (75 nm) sections were prepared from tissue blocks with fibers oriented longitudinally (four to six blocks from each specimen). The ultrathin sections were mounted on copper grids and stained with lead citrate and uranyl acetate. Two grids were made from each of the selected blocks, and one of the grids was randomly chosen for electron microscopy in a JEOL 100S electron microscope (JEOL USA, Peabody, Massachusetts). Six squares of each grid with areas free of nuclei, extracellular space, and microvessels were photographed at a magnification of ×8,000. In addition, the entire grid was scanned, and representative photographs were obtained at magnifications between ×6,000 and ×100,000 for qualitative assessment of the myocardium.

Semithin sections of tissue blocks (four to six blocks per specimen) with fibers arranged in cross section were selected for cell cross-sectional area determinations. Grids of ultrathin sections of these blocks were prepared in the same manner as described above and used with the longitudinal sections for qualitative assessment of the myocardial tissue.

Structural analysis. Total left ventricular volume, cavity volume, and infarct volume were determined by serial reconstruction of integrated cross-sectional areas of the histological sections of each heart. This was done by projecting the heart sections onto a digitizing tablet interfaced with a Zeiss Videoplan image-analysis microcomputer (Carl Zeiss, Thornwood, New York). The left ventricular epicardial and endocardial contours were traced; the infarct zone, if present, was delineated histologically; and the specified cross-sectional areas and volumes were computed. Infarct size was expressed as a percentage of total left ventricular volume. Expansion was graded as described previously: 0, no expansion; 1+, mild thinning of the infarct zone; 2+, mild thinning and dilatation; 3+, moderate thinning and dilatation; and 4+, marked thinning and dilatation.

Further structural analysis was performed on the infarct hearts with expansion and the control hearts. The transverse section of each heart showing maximum increases in infarct thinning and cavity size was used. This section was located within the middle third of the long axis in all hearts. In the hearts measured for cell cross-sectional area and sarcomere length, the histological section analyzed was from the bottom face of the 1-mm slice that had been aposed to the top face of the slice from which the plastic-embedded specimens were obtained. The selected transverse left ventricular sections were divided into six regions by extending radii of cur-

FIGURE 3. Diagram showing expected changes in the value of the form index defined in the text that would result from bulges in the endocardial and epicardial contours. A bulge would cause rounding of the irregular endocardial contour increasing the value of the form factor. A bulge in the curvilinear epicardial contour would make it less round, thus causing a decrease in the value of the form factor.
vature from the center of gravity coordinates of the cavity to the endocardial surface of 1) the region of maximum thinning in the infarct zone, 2) the posterior border zone inside the infarct, 3) the posterior border zone outside the infarct, 4) the midseptal area, most remote from the infarct, 5) the anterior border zone outside the infarct, and 6) the anterior border zone inside the infarct (Figure 2). Equivalent topographic regions were defined in control hearts without infarcts. Wall thickness was determined in each of the six regions by extending their respective radii to the epicardial surface and measuring the distance between the two surfaces. The border regions were defined so that the zones inside the infarct contained only necrotic tissue and those outside contained only normal tissue.

To provide further assessment of the early ventricular shape changes of infarct expansion, endocardial and epicardial shape (form) indexes and maximum contour diameters were calculated and compared within and among groups. The endocardial and epicardial form indexes were defined as

$$4\pi \cdot \frac{\text{Area}}{\text{Perimeter}^2}$$

where Area and Perimeter are defined by either the endocardial or epicardial digitized contour, respectively. This index has the value of 1 when the contour is a circle and approaches 0 as the contour becomes more irregular. A bulge in the usually highly irregular endocardial surface would increase the endocardial form index and maximal endocardial diameter, whereas a bulge in the usually smooth curvilinear epicardial surface would decrease the epicardial form index but increase the epicardial diameter (Figure 3).

**Assessment of changes in cellular dimension and arrangement.** Three methods were used to distinguish the components of stretch and slippage within each region. In the first method, the number of cells across the wall at each site was counted with a light microscope interfaced with the digitizing tablet by a camera lucida as described previously. A grid reticle eyepiece was used to divide the wall thickness into arbitrary unit lengths of 100 µm. The tablet’s LED cursor was tracked transmurally from epicardial to endocardial surface. Each time the cursor light crossed a cell, an event counter was triggered by the observer. All counting was performed at a magnification of ×125. The cell counts were tabulated so that both the number of cells crossed per unit length (cell density) and the total number of cells across the wall were determined. Although we assumed myocytes to have a cylindrical shape, they, in fact, frequently branch at their ends. Approximately 11% of myocytes encountered were at or near branch points, and we used a method described previously to correct for branching. The cell counts were performed twice for each region. In most cases, identical counts were obtained; however, if the two counts differed by more than 5%, cells were recounted another two times. When only two counts were performed, the mean values for cell density and total cells across the wall were used. When repeat counting was necessary, the mean values of the three closest counts were used (repeat counting always resulted,
in three of four counts being within 5% of each other). In addition to acquiring the mean transmural cell density, mean cell density measurements were also compared in the epicardial third, midmyocardial third, and endocardial third of each region to assess possible differences across the wall caused by changes in fiber orientation. If cell stretch alone occurred in a given region, the total number of cells across the wall would not change, but the average cell density across the wall would increase. If bundle slippage alone occurred, transmural cell number would decrease, but cell density would stay constant (Figure 1).

Because cell density would also change if there were a change in the space between cells, as a second independent method of assessing cell stretch, cell cross-sectional area measurements were made on the plastic-embedded semithin sections stained with toluidine. This method distinguished increases in cell density attributable to changes in myocyte dimension from decreases in the intercellular space. Because some myocytes within infarcts have lost their nuclei by 2 days after infarction, cross-sectional area measurements were limited to the 1-day infarcted hearts and controls. For these hearts, 100–150 cross-sectional area determinations were made in each region. Only sections of tissue blocks with cells oriented vertically in cross section were used for measurement. The cross-sectional areas were measured with the light microscope, the camera lucida, and the digitizing tablet of the Zeiss Videoplan computer. Measurements were made across the midportion of each cell at the level of the nucleus at a magnification of $\times 500$.

The third method of assessing myocyte stretch was the measurement of sarcomere lengths in longitudinal myocyte profiles. Six electron micrographs of myocytes in long section were obtained from each heart at a final magnification of $\times 8,000$. Each micrograph contained 30–50 measurable sarcomeres. Sarcomere lengths were measured directly by placing the electron micrographs on the digitizing tablet of the Zeiss Videoplan computer. For the purpose of comparing the contributions of cell dimensional changes to changes in intercellular space, we assumed that myocytes have a cylindrical shape and change little in volume (V) during the 1st day after myocardial infarction. We also assumed that changes in average cell length (l) could be estimated by changes in average sarcomere length. Thus, if

\[ V = A \cdot l \]

where A is cross-sectional area, then cell (and sarcomere length) will vary inversely with cross-sectional area:

\[ l = \frac{V}{A} \]

Values for cell radii (r) were derived from the cross-sectional area by

\[ A = \pi \cdot r^2 \]

or

\[ r = \sqrt{\frac{A}{\pi}} \]

The contribution of the intercellular space (ICS) to cell density was calculated by determining the number of each 100 $\mu$m occupied by cells and subtracting this from 100:

\[ ICS = 100 - (d \cdot \text{density}) \]

where density is measured as cells per 100 microns and $d$ is calculated cell diameter ($2 \cdot r$).

The three methods of assessing cell stretch were complementary. Cell density was used as a parameter estimating stretch because it could be measured at low magnification. This allowed visualization of large fields of myocardium so that identical topographic location was ensured for the entire transmural counting procedure. Measurement of cell cross-sectional areas and sarcomere lengths sacrificed the ability to accurately assess stretch transmurally because higher magnifications with reduced fields of view were necessary. But these methods added sensitivity to the detection of cell stretch in individual myocytes and distinguished myocyte stretch from changes in the intercellular space.

Human heart studies. Histological sections were studied from the hearts of five patients who died within 48 hours of acute myocardial infarction and from two patients without myocardial infarction or significant coronary artery disease. The sections were obtained from fully transmural blocks of ventricular wall. In three infarct hearts, two sections were obtained; one within and the other remote from the infarct zone. In two of the infarct hearts, infarct size was too large to obtain a normal section remote from the infarct region. Thus, transmural sections from the two hearts from patients without myocardial infarction were studied so that a total of five transmural sections from within a thinned infarct region and five sections from normal regions were analyzed. Wall thickness measurements and counting of myocytes across the wall were performed in a manner similar to that described for the rat hearts. The morphometric analysis was limited to transmural cell counts. Comparison between hearts of intercellular and cellular dimensions would not be accurate because the hearts were prepared by immersion fixation without knowledge of their configuration relative to the cardiac cycle.

Statistical methods. Repeated measures analysis of variance19 with region as the repeated measure was used to assess regional differences in wall thickness, cell density, and the number of cells across the wall among the experimental rat groups. The independent and interactive effects of site with age of infarction and severity of expansion were evaluated with respect to each of these measurements. Specifically, the pattern of variation of these
parameters from region to region was examined in control hearts and compared with hearts from rats with infarct expansion, grouped by age of infarction or by extent of expansion. Within regions, quantitative differences were compared between control hearts and infarct expansion hearts. When indicated, the repeated measures analysis of variance was performed on logarithmic transformations of the data to correct for unequal variances between groups. Because comparison of the results with nontransformed and logarithmically transformed data did not affect the significance levels, all reported data is of nontransformed values. One-way analysis of variance was used to compare between-group differences in cavity size and form indexes. When significant differences were found, comparisons between individual groups were made with the Newman-Keuls multiple range test. Regression analysis with a general linear model was used to assess the relation of stretch and slippage with regional wall thickness changes. Regression analysis was also used to correlate changes in cell density with cell cross-sectional area changes. For all statistical analyses, a two-tailed \( p < 0.05 \) was considered significant. The results are reported as the mean ± SEM for values within individual groups; for between-group comparisons, the mean values and the overall standard deviation (square root of error mean square) are reported.

**Results**

**Rat Studies**

**Gross morphological features of hearts.** Of the 60 rats that underwent surgery, 55 survived to the designated times of 1, 2, and 3 days. The other five rats died within the first 6 hours after surgery. Among the surviving animals, 45 had successful coronary ligation resulting in transmural myocardial infarction, and two had small nontransmural infarctions. The eight animals without infarcts served as sham-operated controls. Twenty-eight of the 45 animals with transmural infarctions developed infarct expansion and were used with the eight control animals for data analysis. Hearts of animals that did not develop infarct expansion were excluded from analysis (the two animals with nontransmural infarction and 17 animals with transmural infarction). Twelve of the infarct expansion hearts had 1+ severity, 11 had 2+ severity, and five had 3−4+ severity. Infarct size among the expansion hearts averaged 39 ± 3% and was not significantly different among the hearts grouped either by age of infarction or by degree of expansion. Similar to previous studies, the extent of expansion increased with time. At day 1, seven of nine infarcts had grades of 1+ expansion, whereas five of eight of the day 2 infarcts and nine of 11 of the day 3 infarcts were graded 2+ or greater.

**Changes in ventricular shape.** As we have seen previously, both control hearts and those with

![Figure 5. Plots of comparison of mean regional wall thickness (Panel A) and number of cells across the wall (Panel B) in control rat hearts and infarct expansion rat hearts grouped by extent of expansion. There was progressive wall thinning (\( p < 0.004 \)) and decrease in transmural cell number (\( p = 0.007 \)) as expansion grade increased. However, the pattern of variation in wall thickness and transmural cell number was similar for all expansion grades. Overall standard deviations were the same as in Figure 4.](http://circ.ahajournals.org/)

![Figure 6. Transverse section from middle third of long axis of control rat heart. Note arrangement of muscle bundles of left ventricular (LV) wall. Fiber bundles and cleavage planes separating them (arrow) are roughly perpendicular to the endocardial surface of LV cavity. This arrangement of subendocardial fibers makes the endocardial contour highly irregular. RV, right ventricle; original magnification, \( \times 9 \).](http://circ.ahajournals.org/)
TABLE 1. Morphological Features of the Rat Hearts

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<th>Control (n=8)</th>
<th>1+ expansion (n=12)</th>
<th>2+ expansion (n=11)</th>
<th>3–4+ expansion (n=5)</th>
<th>ANOVA p</th>
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<tr>
<td>Cross-sectional cavity area (mm²)</td>
<td>11.4±1.6</td>
<td>15.7±1.8</td>
<td>22.9±1.8*</td>
<td>28.6±4.2†</td>
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<td>Cavity volume (mm³)</td>
<td>82.7±9.2</td>
<td>97.4±10.0</td>
<td>144.2±10.4*</td>
<td>215.2±20.7†</td>
<td>&lt;0.001</td>
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<tr>
<td>Endocardium</td>
<td>0.36±0.04</td>
<td>0.52±0.04†</td>
<td>0.64±0.04*</td>
<td>0.77±0.03†</td>
<td>&lt;0.001</td>
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<tr>
<td>Epicardium</td>
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<td>0.90±0.01</td>
<td>0.90±0.01</td>
<td>0.91±0.01</td>
<td>0.351</td>
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<td>Maximum diameter (mm)</td>
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<tr>
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<td>5.8±0.3*</td>
<td>7.2±0.3†</td>
<td>&lt;0.001</td>
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*Different from control hearts and 1+ infarct expansion hearts; †different from control hearts and 1+ and 2+ infarct expansion hearts; and ‡different from control hearts (significant differences [p < 0.05] by Newman-Keuls multiple range test).

Infarct expansion showed variation in wall thickness from region to region (p<0.005; Figures 4A and 5A), but the pattern of this regional variability (i.e., left ventricular shape) was different between control hearts and hearts with infarct expansion (p<0.003). Most of the shape change was attributable to infarct thinning; however, all other regions in the hearts with expansion showed significant wall thinning compared with the same regions in control hearts (p<0.0001). There was progressive wall thinning with increasing severity of expansion in all regions, even those remote from the infarct (p<0.004); although wall thinning was greater in the rats killed 3 days after infarction than in those killed after 1 day, when the extent of expansion was controlled for, infarct age was not an independent factor in the severity of thinning.

Cavity size progressively increased as regional wall thickness decreased within and outside of the infarct zone (Table 1). As with wall thickness, cavity dilatation was disproportionately distributed with the greatest increase in radius of curvature

FIGURE 7. Panel A: Transverse section from middle third of long axis of rat heart with large 1-day infarction showing 2+ expansion. Borders of infarct are marked by arrows. Infarct region is thinned and dilated, and overall cavity size is increased. In the septum, inner fiber bundles and cleavage planes have same perpendicular alignment with endocardial surface as was seen in control hearts (original magnification, ×9). Panel B: Portion of septum enclosed at higher magnification (original magnification, ×50). Within infarct zone, angle of inner muscle bundles and cleavage planes relative to endocardial surface is more acute, as can be seen in greater detail in higher magnification view of the boxed region of the infarct (Panel C) (original magnification, ×50). This translation of muscle bundles smooths the endocardial contour. RV, right ventricle; LV, left ventricle.
located in the center of the infarct zone. The changes in ventricular shape resulting from the wall thinning and cavity dilatation were most evident along the endocardial surface of the ventricle. The highly irregular contour of the left ventricular endocardial surface seen in control hearts (Figure 6) became progressively smoothed as the extent of expansion increased. This smoothing of the endocardial surface was mediated by translation of the subendocardial muscle bundles so that the bundles and cleavage planes between them shifted from a comparatively perpendicular orientation relative to the endocardial surface (Figure 6) to a more acute angle (Figure 7). In hearts with mild expansion, this shift in cleavage plane orientation was confined largely to the infarct zone and was most pronounced in the area of greatest thinning (region 1). In hearts with more severe infarct expansion, the shifting of subendocardial muscle bundles occurred progressively in all regions and correlated with the extent of regional wall thinning. The changes in epicardial surface contour were far less dramatic. Quantitatively, the changes in ventricular shape were expressed as significant increases in endocardial (cavity) diameter and endocardial form factor as the extent of expansion increased without significant changes in either epicardial diameter or form factor (Table 1).

General histological and ultrastructural features of infarct zone. Light microscopic evaluation revealed the typical findings of coagulation necrosis. In day-1 hearts, the infarct zone was identifiable by the presence of hypereosinophilia. There was no evidence of intramyocardial rupture or disruption of individual myocytes. By 2 days, the infarcts showed inflammatory infiltration and myocyte degeneration at the lateral margins of the infarct zone. Within the center of the day-2 infarcts, many cells lacked nuclei, but again there was no evidence of cellular disruption. At 3 days, the inflammatory infiltration and myocyte degeneration was more extensive and had moved from the border areas toward the center of the infarct.

The ultrastructural studies confirmed that intramyocardial rupture did not occur within the infarct zone of the hearts with infarct expansion. Although overall cellular integrity was maintained, there was marked intracellular swelling, and numerous breaks were present in the sarcolemma. Some intercalated disks showed a slight widening of the intercellular space, but no complete separation of myocytes at the disks was seen. The lateral intercellular space

![Figure 8](image_url)
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FIGURE 9. Electron micrograph from infarct zone of rat heart 1 day after coronary ligation showing insertion site of large collagen tendon (CT). These collagen tendons connect bundles of individual myocytes. As in Figure 9, this myocyte shows evidence of irreversible ischemic injury. Sarcolemma is lifted off the cell (double arrows), and swollen mitochondria (M) have amorphous densities (single arrows). Original magnification, ×15,000.

was widened between some adjacent cells, but collagen struts linking myocytes within bundles were still present (Figure 8). However, many microvessels were collapsed, which decreased the space between cells with interposing capillaries. The tendonlike collagen connections between myocyte bundles were also present (Figure 9). These larger collagen cables at times appeared to be disrupted, but this effect could also have been caused by passage of the bundles out of the plane of sectioning. The nuclei had marked margination and clumping of chromatin. Mitochondria were swollen with disrupted cristae and had the typical amorphous densities associated with irreversible ischemic injury. Prominent I-bands indicative of sarcomere stretch were present in most cells, but some cells had regions with hypercontracted sarcomeres (see below).

Changes in cellular arrangement and dimension. Number of cells across the wall. Mean transmural cell number plotted by region in control hearts and infarct expansion hearts grouped by age of infarction and extent of expansion, respectively, are shown in Figures 4B and 5B. Transmural cell number changed across regions in both control hearts ($p = 0.008$) and hearts with infarct expansion ($p < 0.0001$; grouped by either age of infarction or severity of expansion). When the shape of the cell number curves is compared with the shape of the regional wall thickness curves in Figures 4A and 5A, they are seen to be

FIGURE 10. Plot of correlation of wall thickness with number of cells across wall for all six regions in control and expansion rat hearts. Solid line represents predicted values derived from linear regression equation. Individual points include all expansion grades. A similar strong correlation was seen when each region and infarct age group was analyzed separately.
practically identical. As with wall thickness, the pattern of cell number variation from site to site was different between control hearts and infarct expansion hearts ($p<0.0001$) but was not significantly different among the expansion groups. There was also a parallel progression of decreased cell number accompanying the decrease in wall thickness seen with expansion. That is, the number of cells across the wall in each region decreased from control hearts to expansion hearts ($p<0.0001$; Figure 4B), and this decrease in cell number progressed with the severity of expansion ($p=0.007$; Figure 5B). Indeed, wall thickness correlated well with cell number in all regions for all hearts ($r=0.915, p<0.001$; Figure 10). A similar strong correlation was seen when each region or each experimental group was analyzed separately.

**Cell density.** Mean myocyte density was not significantly different from region to region in control hearts (Figure 11). In addition, there was no significant difference in the myocyte density from epicardium to endocardium in any given region in either the control or infarct hearts. However, in infarct expansion hearts, an increase in cell density from a mean control value of $5.0 \pm 0.1$ to $6.0 \pm 0.3$ cells/100 $\mu$m ($p<0.0001$) was seen in the region of greatest thinning within the infarct zone (Region 1).

There was no progression of these changes in cell density with infarct age or with severity of expansion. That is, an increase in cell density occurred in the region of maximal thinning in all infarcts with expansion, but the extent of the rise in density did not increase with greater thinning. Moreover, among hearts with expansion, cell density values in the other regions were not significantly different from controls. In the regions away from the site of maximal thinning, a decrease in the number of cells across the wall accounted for all of the change in wall thickness.

**Cell cross-sectional area.** To assess the contribution of cell stretch to the increase in cell density within the infarct zone, myocyte cross-sectional areas of day-1 infarct expansion hearts were compared with control cross-sectional areas. The increase in mean cell density corresponded to a decrease in mean cell cross-sectional area from $238.8 \pm 4.2 \mu^2$ in control hearts to $212.2 \pm 4.3 \mu^2$ in the day-1 infarct expansion hearts ($p<0.001$). Furthermore, the increase in cell density in the infarct zone of each heart was highly correlated to the decrease in cell cross-sectional area ($r = -0.90, p = 0.002$). However, the decrease in cell cross-sectional area within the infarct zone of hearts with expansion was only 11.1% relative to that of control hearts. Assuming that myocyte shape is approximately cylindrical, an 11.1% decrease in cell cross-sectional area would result in an average decrease in cross-sectional diameter of 5.7%. Such a decrease in myocyte diameter would account for only a 5.3% change in cell density if there were no concomitant changes in the intercellular space. This compares with a measured 20% increase in cell density. Thus, cell stretch would appear to contribute only part of the measured change in cell density, the remaining change being caused by a change in the intercellular distance between cells. To confirm this finding, sarcomere-length measurements were performed as a second measure of cell stretch.

**Sarcomere length.** The ultrastructural measurements verified the presence of cell stretch within the infarct zone but, as with cell cross-sectional area, the changes were too small to account for the entire change in cell density. As stated above, prominent I-bands indicative of sarcomere stretch were seen in most cells within the infarct zone of hearts with infarct expansion. Mean sarcomere length increased 12.9% from $1.811 \pm 0.003$ to $2.045 \pm 0.019 \mu$m ($p<0.001$). However, a much wider distribution of sarcomere lengths was present in the infarct hearts. Sarcomere lengths in the control hearts were tightly and symmetrically distributed around the mean in a fairly Gaussian distribution (Figure 12A). In the infarct zone of the expansion hearts, the distribution was much wider (Figure 12B) and skewed toward higher values. As with cell density and cross-sectional area outside of the infarct zone, sarcomere lengths did not significantly differ between expansion and control hearts.
Again assuming a cylindrical shape for myocytes and further assuming no significant loss of cell volume, the 12.9% increase in sarcomere length is very close to the 12.5% increase that would be predicted by the 11.1% decrease in myocyte cross-sectional area. Thus, measurement of sarcomere lengths confirms that cell stretch accounts for approximately 25% of the increase in cell density. Therefore, the remaining change in cell density is caused by a decrease in the distance between cells.

The summation of effects producing the increase in cell density accounts for only a small change in wall thickness. The 20% increase in cell density occurred in the area of maximal infarct thinning but was significantly less predictive of wall thickness changes in this region. The importance of rearrangement of groups of myocytes in determining the extent of wall thinning can be demonstrated by comparing the magnitude of wall thinning expected in the situation of changes in cell density occurring without changes in the number of cells across the wall or the situation of the number of cells across the wall decreasing without changes in cell density and then comparing these expected results with the actual measurements obtained. For example, the mean wall thickness in region 1 of day-1 expanded infarct hearts was 1.1 (±0.1) mm, or a decrease of 1.3 mm from the mean control value of 2.4 (±0.2) mm. If cell density changes alone occurred (by either cell stretch or decreased intercellular space), transmural cell number would not change from the mean control value of 122 (±7), but mean cell density would have to increase from the control value of 5.0 (±0.1) to 11 cells/100 µm—an increase of 120%—to account for the 1.3-mm decrease in wall thickness. But, the observed increase was only 20% to 6.0 (±0.3) cells/100 µm. This 20% increase in cell density accounts for only a 17% decrease in wall thickness if the number of cells across the wall is unchanged. On the other hand, if myocyte rearrangement alone occurred with no change in cell density (5 cells/100 µm), transmural cell number would be expected to decrease from 122 to 57 cells to account for the observed 1.3-mm decrease in wall thickness.
wall thickness. This theoretical calculation based on an assumption of rearrangement of myocyte bundles alone is very close to the actual mean transmural cell count of 65.6 (±4.6) cells in the infarct region for day-1 expansion hearts. A similar analysis can be done in the other regions to demonstrate that changes in the number of cells across the wall (i.e., slippage of groups or bundles of myocytes) accounts for the wall thinning. Table 2 shows the predicted and actual results of this analysis for the infarct zone, and Table 3 shows the results for the remote normal septum in hearts with day-1 infarcts.

Human Heart Studies

As in the rat, there is a striking correlation between the number of cells across the wall and wall thickness (r = 0.94, p < 0.001; Figure 13) in human hearts. Moreover, with calculations similar to those used to construct Tables 2 and 3, the decrease in the number of cells across the wall from a mean of 459.4 (±19.2) cells in normal regions to a mean of 272.8 (±9.5) cells in infarct regions is very close to the predicted 265 cells across the wall in the infarct zone if changes in cell number alone accounted for all wall thinning (from 11.97 ± 0.51 mm in control regions to 8.87 ± 0.26 mm in the infarct region). As in the rat, the changes in wall thickness appeared to be mediated by translation of myocardial fiber bundles so that the cleavage planes relative to the endocardial surface assumed a more acute angle (Figure 14).

Discussion

Infarct expansion, a localized distortion in cardiac shape, affects the geometry and structure of adjacent and remote normal regions. When disproportionate thinning and dilatation occur in the infarct region, they are accompanied by a distortion in shape of the entire heart, including distant normal myocardium. The results show that the structural changes of infarct expansion in rat and human hearts are for the most part mediated by rearrangement of myocytes across the wall. All changes in wall thickness seen with expansion, either within or outside the infarcted region, were accompanied by similar changes in regional transmural cell number. This decrease in the number of cells across the wall was seen in the day-1 rat infarcts as well as in the human and older rat infarcts. Because there was no loss of cell mass at this early time period after infarction, cell dropout could not account for the close correlation of transmural cell number with wall thickness. This implies that the gross remodeling that occurs with infarct expansion is primarily a result of myocyte bundle rearrangement.

We chose to study the mechanism of wall thinning seen with infarct expansion in the rat for several reasons. First, the rat infarct model provides a setting in which to minimize animal-to-animal variation. Animal and heart size, extent of collateral circulation, and other factors that influence basal hemodynamic parameters, infarct size after occlusion of a vessel, myocardial cell size, and microcirculation are kept reasonably reproducible among animals. For this reason, numerous investigators have used the rat to delineate the sequence of

<table>
<thead>
<tr>
<th>Table 2. Actual and Predicted Changes From Control Values of Cell Density and Number of Cells Across the Wall, in the Infarct Region (Region 1), of 1-Day Infarct Expansion Rat Hearts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actual control hearts</td>
</tr>
<tr>
<td>Wall thickness (mm)</td>
</tr>
<tr>
<td>Cell density (cells/100 μm)</td>
</tr>
<tr>
<td>Number of cells across the wall</td>
</tr>
</tbody>
</table>

First column shows actual mean values for wall thickness, cell density, and number of cells across the wall for Region 1 of control rat hearts. Fourth column shows actual mean values of these measurements in rat hearts with 1-day infarcts and expansion. Second column shows predicted values of cell density and the number of cells across wall for the same change in wall thickness if cell density changes alone occur, and the third column shows predicted values if changes in the number of cells across wall alone occur. Percent change from control values is shown in parentheses. Actual decrease in the number of cells across wall is close to the decrease that would be necessary to explain the entire change in wall thickness. Cell density changes account for comparatively little thinning in the infarct zone.
Table 3. Actual and Predicted Changes From Control Values of Cell Density and Number of Cells Across the Wall, in the Remote Septum (Region 4), of 1-Day Infarct Expansion Rat Hearts

<table>
<thead>
<tr>
<th></th>
<th>Actual control hearts</th>
<th>Predicted if cell density changes only</th>
<th>Predicted if cell number changes only</th>
<th>Actual expansion hearts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wall thickness (mm)</td>
<td>2.2</td>
<td>1.7 (↑ 23%)</td>
<td>1.7 (↑ 23%)</td>
<td>1.7 (↑ 23%)</td>
</tr>
<tr>
<td>Cell density (cells/100 μm)</td>
<td>5.0</td>
<td>6.5 (↑ 30%)</td>
<td>5.0 (0%)</td>
<td>5.0 (0%)</td>
</tr>
<tr>
<td>Number of cells across the wall</td>
<td>110</td>
<td>110 (0%)</td>
<td>85 (↑ 23%)</td>
<td>80 (↓ 27%)</td>
</tr>
</tbody>
</table>

As in Table 1, the first column shows actual mean values from control rat, and the fourth column shows actual mean values for infarct expansion rat hearts, but here for the remote septum. Again, the second and third columns show respectively predicted values that would be expected if cell density changes alone or changes in the number of cells across the wall alone accounted for the observed change in wall thickness (percentages shown in parentheses represent the percent change from control values). Actual measured values of cell density and the number of cells across the wall in expansion hearts is very close to what would be predicted if changes in the number of cells across the wall alone occurred. Thus, changes in cell density do not contribute to wall thinning in regions outside of the infarct zone.

gross structural and histological changes that occur with myocardial infarction. Second, although there are minor differences in ventricular structure among various mammalian species, the overall structural features are the same. The principal difference between species with regard to the development of infarct expansion may be the extent of coronary collateral flow to an ischemic region rather than underlying variations in ventricular architecture. The collateral bed in a given species influences infarct size and extent of transmural necrosis. In dogs, which have a rich collateral circulation, coronary ligation rarely leads to large transmural infarctions, and infarct expansion is almost never seen. However, if large transmural infarctions are produced in the dog by embolic occlusion of collateral vessels, an expansion incidence of about 80% is seen. In rats and humans with less-developed collateral circulation, the incidence of large transmural infarctions exhibiting expansion is similar and approaches that of dogs with embolic coronary occlusions. Moderate-to-severe infarct expansion is seen in from one third to one half of anterior transmural infarctions in both rats and humans, while some degree of infarct expansion has been identified in approximately 60–70% of transmural infarcts in rats and in 70% of hearts from humans who have died within 30 days of myocardial infarction. Comparison of experimental studies in rats and dogs with clinical and autopsy studies in humans further indicates that the development of infarct expansion in the three species depends on similar antecedent factors, such as infarct transmurality. In addition, in rats and humans, early infarct expansion is the major contributor to postinfarction dilatation and aneurysm formation. Because of these similarities in incidence, associated factors, and consequences, it seemed reasonable that underlying mechanisms of expansion in rats and humans would also be similar. The closely corresponding results in rat and human hearts in the present study indicates that the mechanism in the two species is likely the same.

The findings of this study are compatible with those of earlier investigations of the cellular changes that occur with nonischemic cardiac dilatation. Ultrastructural studies of sarcomere length have shown that sarcomeres are resistant to overstretch, despite dramatic increases in left ventricular cavity dimension and decreased wall thickness in chronic volume overload. Similar to the findings of the present study, Spotnitz et al reported a strong correlation of wall thickness with transmural cell number in rat hearts passively distended with volume postmortem. They found only a small contribution of changes in cell density to changes in wall thickness. These authors also found a similar translation of the cleavage planes between myocyte bundles with volume-related wall thickness changes. Although not contributing meaningfully to infarct thinning, cell density did increase within the infarct zone, and only within the infarct zone, of expanded hearts. Some of this change in cell density was explained by a decrease in cross-sectional area and an increase in length of the myocytes as determined by cell cross-sectional area and sarcomere-length measurements. Consistent with this, Crozatier et al found sarcomere overstretch within ischemic (not infarcted) segments of canine hearts. Also similar to the results of the present study, these investigators found much greater heterogeneity of sarcomere lengths within the infarct zone than in regions outside of the infarct zone. These authors did not, however, correlate the overstretch of sarcomeres with the extent of thinning and did not study changes in transmural cell number. In the present study, cell dimension changes did not account for all of the observed change in cell density. A significant portion of this change in myocyte density was thus caused by a decrease in intercellular space. The decrease in the space between myocytes within the infarct zone of the day-1 infarct animals was likely the result of a balance between decreases in vascular volume and increases in interstitial space caused by edema. Vogel et al have shown that the loss of vascular filling in acute global ischemia can account for
reductions in wall thickness up to 18%. In our study, the increase in cell density in the infarct zone not accounted for by cell stretch would explain a decrease in wall thickness of approximately 12% and seemed to be largely due to the loss in intravascular volume. In a previous study with the same infarct model, we found that the infarct zone increases in water content by about 6–7% in hearts 1 day after infarction, and it is possible that interstitial edema partially prevents the decrease in intercellular space produced by intravascular collapse. Consistent with this possibility is the observation in the present study that the nonvascular space between cells appeared to be slightly increased.

How does slippage of myocytes occur? Within the infarct zone, intramural rupture of necrotic tissue could take place, allowing displacement and redistribution of cells. Such intramural rupture might account for the predisposition of infarcts with expan-
sion to subsequently rupture.\textsuperscript{25} This alone, however, would not explain the slippage that occurs in other regions of the heart, and we were unable to identify either by light or electron microscopy any evidence for intramural rupture within the infarct zone of hearts with infarct expansion. Intramural rupture by shearing of intercellular connections would not be necessary to explain slippage if the structure of the ventricular wall allowed for some movement between groups of cells. The notion that myocytes are arranged in discrete muscle paths or bundles is not new.\textsuperscript{22–29} In the present study, myocytes in the noninfarcted cardiac muscle of both rat and human hearts appeared to be grouped in bundles arranged in a spirallike fashion separated by cleavage planes. The angle of these planes relative to the endocardial surface was near vertical in noninfarcted regions. A similar arrangement of myocardial fibers in transverse sections of the left ventricle has been described previously in numerous mammalian species,\textsuperscript{22} including man.\textsuperscript{32} Slippage between muscle bundles along cleavage planes could thus account for the redistribution of cells across the wall seen with infarct expansion.

Caulfield and Borg\textsuperscript{33} have described a weblike network of collagen around bundles of myocytes. Within the bundles, myocytes are connected by collagen struts, which likely prevent slippage between cells. Only a few long collagen cables connect the web networks of adjacent muscle bundles, and Caulfield and Borg suggest that the length of these interbundle collagen tendons is sufficient to permit slippage between the bundles and to allow ventricular dilatation to occur. In the present study, the angle of the cleavage planes between muscle bundles translated to more acute angles relative to the endocardial surface in regions of wall thinning, suggesting that slippage between adjacent bundles does occur. This arrangement of subendocardial myocyte bundles and their translation along cleavage planes leads to a far greater change in endocardial contour than epicardial contour (Figures 7 and 14, Table 1) so that large changes in cavity volume can occur without much change in external cardiac size.

We did not find evidence of separation of individual myocytes closely linked within bundles by collagen struts and intercalated disks. This supports the notion that slippage of individual myocytes within bundles does not occur. Although we did not quantify the collagen struts between individual myocytes, there was no evident diminution in their number. This is in contrast to the findings of Zhao et al.,\textsuperscript{34} who found that although there was no evident rearrangement of individual myocytes, collagen struts were diminished in postischemic “stunned” but viable reperfused canine myocardium. This difference between these two studies may reflect interspecies differences or differences between permanent occlusion and reperfusion models. Zhao et al also found with scanning electron microscopy that the collagen cables between myocyte bundles were damaged and suggested that the damage to the collagen matrix may contribute to the observed structural and functional abnormalities in stunned myocardium. Consistent with their results was our finding with transmission electron microscopy that some of the collagen cables appeared torn, but this finding will require confirmation with scanning electron microscopy where ultrathin sections are not required and bundles can be traced through space. Nevertheless, the possibility that early damage to the collagen connections between myocyte bundles occurs in infarcted myocardium suggests a possible mechanism for the bundle slippage seen with infarct expansion.

In summary, rearrangement of groups or bundles of myocytes results in a decreased number of cells across the wall and accounts for most of the acute infarct thinning seen with myocardial infarct expansion and for all the thinning of noninfarcted regions. Cell stretch and the decrease in the intercellular space between myocytes occurs only within the infarct region and contributes far less to infarct thinning.

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