Myocyte Cellular Hyperplasia and Myocyte Cellular Hypertrophy Contribute to Chronic Ventricular Remodeling in Coronary Artery Narrowing–Induced Cardiomyopathy in Rats

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Abstract

To determine whether cardiac failure produced by chronic coronary artery stenosis was associated with the activation of myocyte cellular hyperplasia in the myocardium, the changes in number and size of left ventricular myocytes were measured in rats 3 months after surgery. The hypertrophied left ventricle was found to possess 44%, 32%, 49%, and 48% fewer mononucleated, binucleated, trimonucleated, and tetranucleated myocytes, respectively. In contrast, the hypertrophied right ventricle contained 1.49×10^4 more myocytes as a result of a 2.1-fold, 1.4-fold, and 1.4-fold increase in mononucleated, binucleated, and tetranucleated myocytes. Myocyte cell volume was seen to increase 49% and 21% in left and right ventricular myocytes, respectively. The process of myocyte cellular hyperplasia in the right ventricular myocardium was accompanied by capillary proliferation, and these events were responsible for the parallel addition of newly formed cells and capillaries within the wall and mural thickening. Moreover, the in-series insertion of new myocytes contributed to right ventricular dilation after coronary artery stenosis. In view of the fact that extensive myocardial damage and cell loss may have masked the phenomenon of myocyte cellular hyperplasia in the left ventricle, the presence of DNA synthesis in myocyte nuclei was evaluated at 3 days, 1 week, 2 weeks, 1 month, and 3 months after coronary artery stenosis. Bromodeoxyuridine (BrdU) labeling markedly increased in myocyte nuclei of both ventricles, reaching its peak at 1 and 2 weeks. BrdU labeling of nonmyocyte nuclei also increased but mostly at 2 weeks. To exclude that DNA synthesis was not coupled with ploidy formation, ploidy classes in myocytes were measured by flow cytometry and found not to be increased at 3 months after coronary artery narrowing in both ventricles. In addition, it was documented that mitosis occurred in myocytes and that this process was not associated with a change in the number of nuclei in myocytes. In conclusion, myocyte loss, myocyte cellular hypertrophy, and myocyte cellular hyperplasia all contribute to the development of the cardiomyopathic heart of ischemic origin. (Circ Res. 1994;74:383-400.)

Key Words: DNA synthesis • myocyte mitotic division • myocyte number • myocyte size • ploidy formation • cardiac hypertrophy

It is a general contention that myocardial hypertrophy in the adult heart is accomplished by enlargement of preexisting muscle cells with no cell proliferation.1,2 Cardiac myocytes have been claimed to be terminally differentiated cells and have often been compared with neurons for their inability to regenerate and replace damaged myocardium.3 However, observations in humans4-7 and animals8-12 have provided strong supportive evidence that myocyte cellular hyperplasia may occur under a variety of pathological conditions characterized by a large and prolonged stress on the myocardium. In contrast, studies aiming at the identification and quantification of DNA replication in the overloaded myocardium have documented no labeling13 or little DNA synthesis in myocyte nuclei, possibly representing polyploid cells.14,15 These opposing results may be related to differences in the various animal models and the presence or absence of ventricular dysfunction and failure. Impairment of cardiac pump function appears to be a consistent factor for the activation of myocyte proliferation.4,9,12-16 In particular, diastolic Laplace overloading has been shown to be related to this cellular response, whereas modifications of systolic wall stress have a lesser effect on this cellular process.18 Ischemic cardiomyopathy produced by nonocclusive coronary artery constriction is characterized in both humans and animals by chronic loss of myocytes, cardiac hypertrophy, and myocardial scarring that lead to ventricular dilation and increased diastolic wall stress.19 The functional impairment of the left ventricle may also affect the compliance properties of the right ventricle, and this phenomenon may account for diastolic overload and eccentric right ventricular hypertrophy.19-22 Recently, it was shown that shortly after coronary stenosis, DNA synthesis was present in left and right ventricular myocytes in combination with the appearance of nuclear mitotic division.23 Although these findings strongly suggested that myocyte cellular hyperplasia may participate in the remodeling of the ventricles, no definite evidence was provided for this contention. In the present study, therefore, a complex methodological approach was used to demonstrate unequivocally whether myocyte cellular hyperplasia is an important component of the restructuring of the ventricular wall.

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during the acute, subacute, and chronic phases of the cardiac myopathy generated by coronary artery constriction in rats.

Materials and Methods

Animals

Experiments were carried out in male Sprague-Dawley rats at 2 months of age (Charles River Breeding Laboratories, North Wilmington, Mass). Coronary artery narrowing was performed in 114 animals. Fifty-nine animals in this group died during the 3-month period of experimentation, so 55 rats were included in the study. Twenty-five sham-operated (SO) rats served as controls. Animals were killed from 3 days to 3 months after surgery. Protocol details are described in each specific section.

Coronary Artery Narrowing

Under ether anesthesia, thoracotomy was performed, the left coronary artery located, and a suture positioned around the vessel 1 to 2 mm from its origin. Subsequently, a probe 275 \( \mu \text{m} \) in diameter was held in contact with the wall of the exposed coronary artery. The entire vessel and the probe were ligated, and the probe was quickly removed. The chest was closed, and the animals were allowed to recover. Sham-operated control rats were treated similarly except that the ligature around the coronary artery was not tied.

Functional Measurements

Just before they were killed, animals were anesthetized with chloral hydrate (300 mg/kg body weight IP), and the external right carotid artery was exposed and cannulated with a microtip pressure transducer catheter (Millar PR 249; diameter, 0.99 mm) connected to an electrostatic chart recorder (Gould ES 2000). After arterial blood pressure was monitored, the catheter was advanced into the left ventricle for the evaluation of left ventricular pressures and dP/dt. Subsequently, a second catheter (Millar PR 249) with a 120° curved tip was inserted into the right jugular vein and advanced through the superior vena cava and the right atrium into the right ventricular chamber for measurements of right ventricular pressures and dP/dt. Subsequently, rats were respirated on room air. A thoracotomy was performed, and an ultrasonic Sistolic flow probe (DBF-110A-CP, 10 MHz, 3.5-mm internal diameter) connected to a pulsed Doppler velocimeter (HVPD-10, Crystal Biotech, Hopkinton, Mass) was placed around the ascending aorta. The flow probe, connected to a pulsed Doppler amplifier operating at 10 MHz, was used in conjunction with the velocimeter to determine cardiac output as previously described.

In Situ Morphometric Studies

Quantitative analysis of the myocardium was performed at the 3-month time point only. Twenty-seven coronary-constricted rats and 12 SO control animals were included in this part of the study.

Fixation Procedure

At the completion of the hemodynamic determinations, animals were killed by arrest of the heart in diastole with 1 mL cadmium chloride (100 mmol/L IV). The heart was then perfused through the abdominal aorta at a pressure corresponding to mean arterial pressure. The left ventricular chamber was filled with fixative and kept at a pressure equal to end-diastolic pressure. After perfusion with pH 7.2 phosphate buffer for 3 minutes, the coronary vasculature was perfused for 15 minutes with a solution containing 2% paraformaldehyde and 2.5% glutaraldehyde. Subsequently, the heart was excised, and the weights of the left and right ventricles were recorded. The volume of each ventricle was determined by dividing its weight by the specific gravity of muscle tissue, 1.06 g/mL.

Degree of Coronary Artery Narrowing

The initial 2- to 3-mm segment of the left coronary artery was dissected free, and the luminal diameters of the vessel adjacent to the narrowed site and in the constricted portion were measured with a dissecting microscope. Maximal and minimal internal diameters in each of these two locations were determined at a magnification of \( \times 20 \), and the geometric mean value was calculated. The degree of constriction was evaluated by comparing the vessel diameter above the stenosis with the diameter at the level of the stenosis.

Tissue Sampling

Each heart was sectioned perpendicular to its longitudinal axis. Two adjacent sections, midway between the base and the apex, were obtained to measure chamber luminal diameter and the thickness of the free wall of the left and right ventricles. Eight equally spaced measurements of the left and right ventricular free wall were collected from each slice, and their values were averaged. Subsequently, the left and right ventricular free walls were cut into small pieces to obtain approximately thirty 1-mm-thick tissue blocks, which were postfixed in osmium, dehydrated in acetone, and flat-embedded in araldite. These samples were used for the light microscopic estimations of the numerical density of myocyte nuclei, myocyte nuclear length, and electron microscopic morphometry (see below). Two large additional slices of each ventricle were also processed in a similar manner and used for the quantitative light microscopic evaluation of tissue damage in the myocardium (see below).

Morphometric Determination of Myocyte Damage

Sections 1 to 2 \( \mu \text{m} \) thick were cut with a glass knife 38 mm long (model 2078, Histoknife Maker, LKB Instruments, Gaithersburg, Md) and a rotary microtome (HM 350, Microm, Carl Zeiss, Inc, Thornwood, NY). These sections, which contained the entire thickness of the wall, were stained with toluidine blue. Fifteen consecutive fields each from the endomyocardium and epimyocardium in each animal were examined at a calibrated magnification of \( \times 400 \) with a reticle containing 42 sampling points (105844, Wild Heerbrugg Instruments, Inc, Farmingdale, NY). This reticle defined a tissue area of 62.500 \( \mu \text{m}^2 \), and the fraction of points lying over foci of replacement fibrosis was measured to compute the volume fraction of lesions in the myocardium of each ventricle.

Morphometric Determination of Myocyte Nuclear Density and Myocyte Cell Volume per Nucleus

Ten randomly chosen tissue blocks from each ventricle were sectioned at a thickness of 0.75 \( \mu \text{m} \) and stained with toluidine blue. In transversely sectioned muscle fibers, the number of myocyte nuclei, \( N(n) \), included in an area of 9950 \( \mu \text{m}^2 \), A, was counted (\( \times 1000 \)). A total of 20 to 25 fields were evaluated in the endomyocardium and epimyocardium of each ventricle of each animal to determine the number of nuclear profiles per unit area of tissue, \( N(n)_v \), and the volume fraction of myocytes, \( V(m)_v \), in the myocardium. Nuclear length, \( D(n) \), was determined in the endomyocardial and epimyocardial layers of each ventricle from 50 measurements made at a magnification of \( \times 1250 \) in longitudinally oriented myocytes. Ten blocks with myofibers sectioned parallel to their length were cut, sections approximately 2 \( \mu \text{m} \) thick were collected and stained, and 10 measurements of nuclear length were recorded from each tissue section, 5 each for each region of the wall. From the quotient of \( N(n)_v \) and \( D(n) \), the number of myocyte nuclei per unit volume of myocardium, \( N(n)_v \), was computed. Myocyte cell volume per nucleus in each layer of the wall of each ventricle, \( V(m)_v \), was obtained by dividing the volume fraction of myocytes, \( V(m)_v \), by the number of myocyte nuclei per unit volume of myocardium:

\[ V(m)_v = \frac{V(m)}{N(n)} \]
(1) \[ V(m) = V(m)/N(n) \]

The total number of myocyte nuclei in each ventricle, \( N(n)_T \), was then derived from the product of the number per unit volume, \( N(n)_V \), and the total ventricular volume, \( V_T \):

(2) \[ N(n)_V = N(n)_T \times V_T \]

The quantitative analysis described here was performed in 12 control and 27 coronary-constricted rats for the right ventricle. The estimations in the left ventricle were restricted to 12 control and 13 coronary artery-narrowed (CAN) rats.

Electron Microscopic Morphometry for Computations of Number of Myocytes and Capillaries in the Right Ventricle Wall

In each animal, eight blocks with myofibers oriented in the transverse direction and an additional two blocks with longitudinally oriented cells were used. Low-power electron micrographs of transverse sections of myocardium, seven from each tissue block, were collected and printed at \( \times 5000 \). These micrographs were analyzed with a superimposed grid consisting of 140 sampling points and 14 test-line segments.28

The volume fraction of myocardial components was measured in 2184 of these low-power micrographs, 672 from sham-operated controls and 1512 from CAN animals, by counting the fraction of sampling points overlying myocytes, capillaries, and the remaining portion of the interstitium. The numbers of myocytes and capillary profiles in the sampled area were counted to estimate their numerical densities and average cross-sectional areas.29

The mean center-to-center distance 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 pattern.9,27,28,30,31 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. On this basis, the transmural number of myocytes, \( N(m)_{m} \), and capillaries across the wall of thickness \( W \) can be found from

(3) \[ N(m)_{m} = W/d = 1.0243 W [N(m)_A]^{1/2} \]

Figure 1. Bar graphs showing effects of coronary artery narrowing (CAN) of 3 months’ duration on the aggregate number of left (A) and right (B) ventricular myocardite nuclei and left (C) and right (D) ventricular myocyte cell volume per nucleus. Results are presented as mean ± SD. *Significantly different, \( P < .05 \). SO indicates sham-operated control animals. SO, \( n=12 \) in both ventricles; CAN, \( n=13 \) in the left ventricle, \( n=27 \) in the right ventricle.

Studies in Isolated Ventricular Myocytes

The analysis of the distribution of nuclei in myocytes was performed in cells isolated from CAN and SO rats at 1 week, 2 weeks, 1 month, and 3 months after surgery. At the 1-week, 2-week, and 3-month time points, three CAN rats each were used, whereas at the 1-month interval, four animals were included. Three SO control rats were studied at each time with the exception of the 1-month period, in which two animals were included.

Myocyte Isolation

Hearts were excised and myocytes were enzymatically dissociated according to a procedure previously described.11,12 In these preparations, rectangular, trypan blue–excluding cells constituted nearly 75% to 85% of all myocytes. The average number of myocytes collected from the left and right ventricles of SO rats was \( 14 \times 10^6 \) and \( 3.5 \times 10^6 \), respectively. Corresponding values in experimental rats were \( 10 \times 10^6 \) and \( 3.0 \times 10^6 \). Surgical interventions and increases in the collagen compart-
Heart wall thickness and myocyte diameter were measured from preparations treated with bisbenzimide H33258. A random sampling of 1000 myocytes from each ventricle in each heart was used to determine the relative frequency of mononucleated, binucleated, and multinucleated cells.

**Incorporation of Bromodeoxyuridine in Myocytes and Nonmyocytes**

Rats were injected with bromodeoxyuridine (BrdU) (Boehringer Mannheim, 50 mg/kg body weight IP) 24 hours before they were killed. Under anesthesia, the heart was removed, and frozen sections 4 to 6 μm thick of each ventricle were obtained. Subsequently, BrdU localization in the cells was detected by immunofluorescence using monoclonal antibody. 

Briefly, myocardial sections were fixed for 30 minutes at −20°C in 70% ethanol/glycine buffer. These sections were then incubated for 30 minutes with anti-BrdU mouse monoclonal antibody (clone BMC 9318) and nuclei at 37°C. Incubation with the secondary antibody (sheep anti-mouse Ig fluorescein-labeled) was also performed for 30 minutes at 37°C. Finally, sections were stained with bisbenzimide H33258 (50 μg/mL) for 15 minutes at room temperature. The percentage of nuclei labeled with BrdU was determined by counting approximately 1000 myocyte nuclei and 1000 nonmyocyte nuclei in the inner, middle, and outer layers of each ventricle in each heart. This analysis was performed in coronary-constricted rats killed 3 days, 1 week, 2 weeks, 1 month, and 3 months after surgery. Three experimental rats were included at each time point, with the exception of the 2-week period, in which four animals were used. Seven SO rats were used as controls.

**Flow Cytometry of Myocyte Nuclei**

Isolated cardiac myocytes were separated from nonmyocytes by centrifugation through Percoll (Pharmacia Fine Chemicals, Uppsala, Sweden). Approximately 10^7 cells were suspended in 10 mL of isotonic Percoll (final concentration, 41% in resuspension medium) and centrifuged for 15 minutes at 34°C. Populations of myocytes were recovered from the pellet, their purity was evaluated, and they were subsequently treated with hypotonic buffer (0.001 mol/L HEPES, 1.5 mmol/L MgCl₂, pH 7.4) for 5 minutes; then lysis buffer (3% glacial acetic acid, 5% ethylhexadecyl(dimethyl ammonium bromide in water) was added, and tubes were shaken every 2 minutes for 10 minutes. By this procedure, myocyte nuclei were fully dissociated from the myocyte cytoplasm. Subsequently, nuclei were washed in phosphate-buffered saline by centrifugation (10 minutes, 1000g), and the nuclear DNA and protein were stained with 10 μg/mL diamidino-2-phenylindole and 10 μg/mL sulforhodamine 101 (Eastman Kodak, Rochester, NY) dissolved in 10 mmol/L piperazine-N,N-bis-2-ethanesulfonic acid buffer (Calbiochem, La Jolla, Calif) containing 100 mmol/L NaCl, 2 mmol/L MgCl₂, and 0.1% Triton X-100 (pH 6.8), as described. The fluorescence of individual nuclei was measured with an IPC-22 flow cytometer (Ortho Diagnostics, Westwood, Mass) using an appropriate dichroic mirror and fluorescence of proteins counterstained with sulforhodamine 101. The data were stored and analyzed using MULTICYTE (Phoenix Flow Systems, San Diego, Calif) on a Compaq 386 personal computer. The DNA frequency histograms were deconvolved, and the results were computer analyzed with MULTICYTE software (Phoenix). The percentages of diploid G₁ and tetraploid G₂ nuclei were determined in each preparation. This analysis involved three CAN and three SO rats killed at 3 months after surgery.

**Detection of Mitosis**

Colchicine was injected intravenously in rats at a dose of 100 mg/kg body weight to arrest cells entering mitosis in metaphase. Animals were killed 4 hours later and frozen sections of the left and right ventricles obtained. Subsequently, these sections were fixed for 15 minutes with 2.5% glutaraldehyde in phosphate buffer and then stained with bisbenzimide H33258 (50 μg/mL).
FIG 3. Frozen sections of left ventricular myocardium illustrating bromodeoxyuridine (BrdU) labeling of nonmyocyte nuclei (A and B) and myocyte nuclei (C and D) 1 week after coronary artery narrowing. Left panels illustrate BrdU labeling by immunofluorescence in nonmyocyte nuclei (A) and in a myocyte nucleus (C), whereas right panels show the same fields by phase-contrast microscopy and bisbenzimide H33258 fluorescence. Magnification ×1100.
Fig 4. Frozen sections of ventricular myocardium showing bromodeoxyuridine (BrdU) labeling of left ventricular myocyte nuclei 1 month (A and B) and three months (C and D) after coronary artery narrowing. Left panels illustrate BrdU labeling by immunofluorescence, whereas right panels show the same fields by phase-contrast microscopy and bisbenzimide H33258 fluorescence. Magnification ×1100.
for 15 minutes at room temperature. Mitotic images in myocytes and nonmyocyte cells were identified by fluorescence microscopy at ×1250 magnification. This analysis was restricted to animals at 1 and 2 weeks after coronary artery narrowing. This part of the study included two SO and five CAN rats.

An additional group of five CAN and three SO rats at 1 month after surgery were fixed by perfusion of the coronary vasculature and embedded in paraffin as described above. These samples were used for the light microscopic detection of mitotic images in myocytes and nonmyocytes in the absence of colchicine injection.

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 for comparisons between two measurements was determined with the unpaired two-tailed Student's t test. Statistical significance for comparisons among measurements within the wall of each ventricle was determined with a one-way analysis of variance. Statistical significance in multiple comparisons among independent groups of data in which analysis of variance and the F test indicated the presence of significant differences was determined by the Bonferroni method.28 Values of P<.05 were considered significant. Because measurements presented were not obtained in all animals, n values for each parameter determined are listed in the text or the legend of each figure.

Results

Global Cardiac Performance

The surgical procedure used for the imposition of nonocclusive stenosis of the left main coronary artery near its origin resulted in reductions in luminal diameter of 50±16%. Such a change in linear dimension corresponded to a 75% decrease in luminal cross-sectional area. These determinations were obtained in the 27 experimental animals killed 3 months after surgery in which coronary vessels were fixed by vascular perfusion. Body weight was similar in SO controls, 495±46 g, and CAN rats, 488±47 g. Measurements of left ventricular performance obtained in 10 SO and 21 CAN rats demonstrated that left ventricular end-diastolic pressure (SO, 6.1±2.1 mm Hg; CAN, 22±5.2 mm Hg) increased 261% (P<.0001) as a result of coronary artery narrowing, whereas left ventricular systolic pressure (SO, 118±5 mm Hg; CAN, 91±11 mm Hg) decreased 23% (P<.0001). Positive dP/dt and negative dP/dt were reduced 37% (P<.0001) and 38% (P<.0001), respectively, after coronary artery stenosis (data not shown). In addition, measurements in 10 SO and 8 CAN animals demonstrated that stroke volume was reduced 38% (P<.0001) (SO, 0.32±0.04 mL; CAN, 0.20±0.03 mL) and cardiac output was depressed 40% (P<.0001) (SO, 124.8±13.9 mL/min; CAN, 74.8±6.8 mL/min) with coronary stenosis. Finally, the analysis of right ventricular function (SO, n=10; CAN, n=21) indicated that right ventricular end-diastolic and systolic pressures were increased 291% (P<.005) and 10% (P<.03) in experimental animals. In contrast, positive dP/dt was reduced 22% (P<.001) and negative dP/dt 24% (P<.001).

In summary, coronary artery constriction of 3 months' duration produced left ventricular failure and right ventricular dysfunction.
TABLE 3

<table>
<thead>
<tr>
<th>% LV myocyte nuclei (71 629)</th>
<th>SO (n=7)</th>
<th>CAN, 3 D (n=3)</th>
<th>CAN, 1 W (n=3)</th>
<th>CAN, 2 W (n=4)</th>
<th>CAN, 1 Mo (n=3)</th>
<th>CAN, 3 Mo (n=3)</th>
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<tbody>
<tr>
<td>Endomyocardium</td>
<td>0.10±0.10</td>
<td>0.09±0.01</td>
<td>0.76±0.19*†</td>
<td>0.70±0.24*†</td>
<td>0.41±0.24</td>
<td>0.10±0.08§</td>
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<tr>
<td>Midmyocardium</td>
<td>0.23±0.16</td>
<td>0.28±0.07</td>
<td>1.25±0.23*†</td>
<td>0.77±0.18*</td>
<td>0.56±0.27†</td>
<td>0.23±0.13‡</td>
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<tr>
<td>Epimyocardium</td>
<td>0.14±0.12</td>
<td>0.42±0.13</td>
<td>1.50±0.32†</td>
<td>0.70±0.21†</td>
<td>0.47±0.31†</td>
<td>0.19±0.08‡</td>
</tr>
<tr>
<td>% RV myocyte nuclei (69 074)</td>
<td>0.11±0.10</td>
<td>0.07±0.05</td>
<td>0.62±0.09†</td>
<td>0.49±0.18†</td>
<td>0.30±0.17</td>
<td>0.13±0.04§</td>
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<tr>
<td>Endomyocardium</td>
<td>0.17±0.18</td>
<td>0.21±0.01</td>
<td>0.73±0.17†</td>
<td>0.54±0.20*</td>
<td>0.42±0.11</td>
<td>0.10±0.01§</td>
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<tr>
<td>Midmyocardium</td>
<td>0.10±0.04</td>
<td>0.16±0.04</td>
<td>0.73±0.06†</td>
<td>0.57±0.30†</td>
<td>0.37±0.06</td>
<td>0.17±0.04‡</td>
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<tr>
<td>% LV nonmyocyte nuclei (76 234)</td>
<td>2.64±0.93</td>
<td>2.07±0.46</td>
<td>4.33±1.55</td>
<td>7.65±1.59**‡</td>
<td>3.06±0.53§</td>
<td>2.47±0.89§</td>
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<td>Endomyocardium</td>
<td>2.84±0.87</td>
<td>2.77±1.13</td>
<td>5.09±0.22</td>
<td>9.16±2.03**‡</td>
<td>3.21±0.63§</td>
<td>2.89±0.48§</td>
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<td>Midmyocardium</td>
<td>2.67±0.80</td>
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<td>2.86±0.65§</td>
<td>1.94±0.62§</td>
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<tr>
<td>% RV nonmyocyte nuclei (74 100)</td>
<td>2.71±0.91</td>
<td>2.58±0.31</td>
<td>2.87±0.18</td>
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<tr>
<td>Endomyocardium</td>
<td>2.76±0.91</td>
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<td>3.61±0.40</td>
<td>6.72±1.78**‡</td>
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<td>2.53±0.80§</td>
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<tr>
<td>Midmyocardium</td>
<td>2.02±0.40</td>
<td>3.10±0.39</td>
<td>4.53±1.47</td>
<td>7.09±1.92**‡</td>
<td>2.32±0.22$</td>
<td>1.54±0.15$</td>
</tr>
</tbody>
</table>

BrDU indicates bromodeoxyuridine; SO, sham-operated; CAN, coronary artery narrowing; LV, left ventricle; and RV, right ventricle. Results are presented as mean±SD. *Statistically significant difference from SO; †statistically significant difference from CAN, 3 days; ‡statistically significant difference from CAN, 1 week; ‡‡statistically significant difference from CAN, 2 weeks. Numbers in parentheses indicate the total number of nuclei counted for each determination.

Cardiac Hypertrophy

Measurements of cardiac weights documented that 3 months after coronary artery constriction, heart weight was increased 22% (P<.0001) in experimental animals (SO, 1214±109 mg; CAN, 1486±168 mg). This change was the result of 12% (P<.005) and 68% (P<.0001) increases in the weight of the left (SO, 991±110 mg; CAN, 1113±115 mg) and right (SO, 223±25 mg; CAN, 373±76 mg) ventricles, respectively. In the left ventricle, wall thickness was decreased 11% (P<.04), from 2.03±0.28 to 1.81±0.22 mm, and chamber diameter was increased 20% (P<.001), from 8.20±0.91 to 9.85±1.10 mm. In the right ventricle, mural thickness increased 31% (P<.0001) with coronary constriction, from 1.01±0.16 mm in controls to 1.52±0.24 mm in experimental animals. In view of the impossibility of measuring chamber diameter in the right ventricle, ventricular wall area was calculated. The computation of ventricular wall area, which is obtained by dividing wall volume by wall thickness, assumes that the ventricular wall may be treated as a thin sheet. Thus, increases in wall area imply large chamber volumes. Right ventricular wall area was increased 28% (P<.005) in CAN rats (SO, 208±41 mm²; CAN, 266±58 mm²).

In summary, coronary artery constriction of 3 months’ duration resulted in myocardial hypertrophy and cavity dilation of both ventricles.

Number of Myocyte Nuclei in the Ventricle and Myocyte Cell Volume per Nucleus

Consistent with previous observations, multiple foci of replacement fibrosis were found across the left and right ventricular walls. In the left ventricle, however, these sites of tissue injury were larger and more numerous. Quantitatively, the volume percent of myocardial fibrosis in the left ventricle was 0.59±0.14% in SO animals and 16.1±10.4% in CAN rats. Corresponding values in the right ventricle were 0.20±0.01% and 2.55±0.54%. The 27-fold and 13-fold increases in tissue damage in the left and right ventricles were statistically significant (P<.0001).

The changes in the total number of myocyte nuclei in the ventricles are illustrated in Fig 1. In comparison with SO animals, CAN rats exhibited a 52% (P<.0001) loss in the total number of myocyte nuclei in the left ventricle (Fig 1A). In contrast, the aggregate number of myocyte nuclei in the right ventricle increased 38% (P<.0001) with coronary stenosis (Fig 1B). Specifically, the left ventricle of experimental animals possessed 14.1±104 fewer nuclei than normal hearts, whereas the right ventricle had 2.7x10^6 more nuclei than controls.

Figs 1C and 1D show the measurements of myocyte cell volume per nucleus in SO and CAN rats. This parameter was not different in the endocardial and epicardial layers of the wall of each ventricle in both animal groups. Therefore, regional values were combined. Coronary narrowing was associated with 49% (P<.0001) and 21% (P<.0001) increases in myocyte cell volume per nucleus in the left and right ventricles, respectively.

In summary, coronary artery constriction of 3 months’ duration resulted in myocardial damage, myocyte cell loss, and myocyte cellular hypertrophy in the left ventricle and myocyte nuclear hyperplasia and myocyte cellular hypertrophy in the right ventricle.
Mural Number of Myocytes and Capillaries

Since myocyte nuclear hyperplasia may be indicative of myocyte cellular hyperplasia and the addition in parallel of newly formed cells within the wall,\textsuperscript{9} such a possibility was examined. In addition, the adaptation of the capillary network was measured because capillary proliferation may parallel myocyte proliferation.\textsuperscript{40} These quantitative analyses were restricted to the right ventricle. Mural thickness and the aggregate number of myocyte nuclei increased in this side of the heart only.

The estimation of the mural number of myocytes and capillaries required the measurement of their numerical densities in the myocardium. Results demonstrated that the number of myocytes per square millimeter of tissue was 3934±626 in SO and 3314±558 in CAN rats. The 16% decrease in this quantity was statistically significant ($P<.005$). Capillary density also decreased 5% with coronary stenosis (SO, 4099±732; CAN, 3879±517), but this change was not statistically different. As a result of these modifications, the center-to-center distance of myocytes increased 9% ($P<.02$), from 17.1±1.9 $\mu$m in SO to 18.7±1.5 $\mu$m in CAN rats. This dimension did not vary for the capillary network (SO, 5.69±0.52 $\mu$m; CAN, 5.98±0.47 $\mu$m).

The availability of myocyte and capillary densities and wall thickness allowed the evaluation of the numbers of myocytes and capillaries included in the wall.\textsuperscript{9,27,31,40,41} Three months after coronary stenosis, wall thickness increased 31% and the transmural number of myocytes 20% ($P<.02$) (Fig 2). Since myocyte diameter expanded 10% ($P<.01$), the increases in myocyte number and in the lateral dimension of these cells both contribute to wall thickening after coronary narrowing. The mural number of capillaries increased 28% ($P<.01$) (SO, 66.2±10.7; CAN, 84.5±16.2) with coronary constriction, but capillary diameter remained unchanged (data not shown). Average sarcomere length did not vary with coronary stenosis (SO, 2.04±0.08 $\mu$m; CAN, 2.07±0.11 $\mu$m).

In summary, coronary artery constriction of 3 months' duration resulted in the parallel addition of newly formed myocytes and capillaries, which, in combination with an increase in myocyte diameter, contributed to the thickening of the right ventricular wall.

DNA Synthesis in Myocyte and Nonmyocyte Nuclei

The morphometric results described above indicated that myocyte nuclear and cellular hyperplasia occurred in the right ventricle after coronary narrowing. However, quantitative analysis of the left ventricle failed to demonstrate an increase in the total number of myocyte nuclei. Conversely, extensive myocardial damage and myocyte cell loss may have masked an actual increase in myocyte nuclei and cells in the left ventricle. Therefore, the incorporation of BrdU in myocyte and nonmyocyte nuclei was measured in both ventricles 3 days, 1 week, 2 weeks, 1 month, and 3 months after surgery.

The light microscopic examination of tissue sections showed that BrdU labeling of myocyte and nonmyocyte nuclei seemed to increase 1 and 2 weeks after coronary narrowing (Fig 3). Although BrdU labeling of all cell populations decreased with time in CAN rats, stained myocyte nuclei (Fig 4) and nonmyocyte nuclei were observed up to 3 months after surgery. The quantitative results illustrated in Fig 5A involved the analysis of
50 598 myocyte nuclei in the endomyocardium, midmyocardium, and epimyocardium of the left ventricle of 16 CAN rats and a total of 21 031 myocyte nuclei collected from the same regions of the left ventricle of 7 SO animals. Preliminary counts indicated that sham operation per se did not affect BrdU labeling over time, so that animals at 1 week (n=2), 2 weeks (n=1), 1 month (n=1), and 3 months (n=3) were combined in a single group of 7 control rats. In comparison with baseline values, coronary artery stenosis was associated with 1.69-fold (P=NS), 7.31-fold (P<.0001), 4.50-fold (P<.002), 3.00-fold (P=NS), and 1.06-fold (P=NS) increases in the percentage of BrdU-stained myocyte nuclei in the left ventricular wall at 3 days, 1 week, 2 weeks, 1 month, and 3 months, respectively. The major increase in this parameter was seen at 1 week, progressively decreasing to control values with time. In addition, similar values in the three layers of the wall were seen at all intervals (Table 1).

Fig 5B shows BrdU labeling of myocyte nuclei in the right ventricle. For this analysis, 48 249 and 20 825 myocyte nuclei were counted in CAN and SO animals, respectively. Coronary stenosis was associated with 5.31-fold (P<.001) and 4.08-fold (P<.002) increases in the percent labeling of myocyte nuclei at 1 and 2 weeks after surgery. No significant changes were detected at 3 days or 1 and 3 months. Moreover, similar levels of labeling were detected in the major layers of the right ventricular wall throughout (Table 1).

The observation that DNA synthesis occurred in myocyte nuclei shortly after coronary narrowing does

Fig 5C illustrates the percent labeling of nonmyocyte nuclei in the left ventricle. For this analysis, 53 361 and 22 873 nuclei were counted in CAN and SO rats, respectively. The fraction of cells stained by BrdU was higher than that of myocytes. Coronary narrowing produced an increased labeling of interstitial cells that was apparent at the 2-week interval. No change was detected at 3 days, and the 90% increase measured at 1 week was not statistically different. At 2 weeks, a 3.02-fold (P<.001) increase was measured in the entire wall. Regionally, 2.94-fold (P<.0001), 3.23-fold (P<.001), and 2.90-fold (P<.001) increases were seen in the epicardium, midmyocardium, and endomyocardium (Table 1). One month and 3 months after surgery, labeling of nonmyocyte nuclei was practically identical to control values. The quantification of this phenomenon in the right ventricle involved the analysis of 22 010 nuclei in SO animals and 52 090 nuclei in CAN rats (Fig 5D and Table 1). The nonmyocyte cell populations in this ventricle showed a response similar to that seen in the left ventricle. BrdU labeling was maximal at 2 weeks, returning to baseline at 1 month and 3 months.

In summary, coronary artery constriction resulted in a marked increase in DNA synthesis in myocyte and nonmyocyte nuclei that was maximal between 1 and 2 weeks after surgery.

**Frequency of Polyploidy in Myocyte Nuclei**

The observation that DNA synthesis occurred in myocyte nuclei shortly after coronary narrowing does
Fig 8. Frozen sections of left ventricular myocardium (A through D) from coronary artery-narrowed rats 1 week after surgery. Animals were injected with colchicine 4 hours before they were killed. Myocyte nuclei undergoing mitosis are illustrated in the left panels by bisbenzimide H33258 fluorescence and in the right panels by a combination of phase-contrast microscopy and bisbenzimide H33258 fluorescence. Arrowheads indicate myocyte nuclei undergoing mitosis. Magnification ×1200.
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Fig 9. Semithin sections of plastic-embedded left ventricular myocardium obtained from rats killed 1 month (A) and 3 months (B) after coronary artery narrowing. A mitotic image in a myocyte is shown in A, whereas the identification of the cell of origin of the mitotic figure shown in B is uncertain. Methylene blue and safranin staining. Magnification: A, ×1600; B, ×2400.

not document whether this DNA synthetic activity was coupled with mitotic division or ploidy formation. Therefore, the frequency distribution of DNA content was measured by flow cytometry in myocyte nuclei isolated from SO and CAN rats 3 months after surgery. This interval was selected because it corresponded to the period at which the number of myocyte nuclei in the myocardium was examined morphometrically. As illustrated in Fig 6, flow cytometry demonstrated that in control and experimental animals, nuclei with a diploid (2N) amount of DNA constituted the major component of the total population. Tetraploid (4N) nuclei represented a small fraction of the population, whereas other categories of polyploid muscle cell nuclei were not observed. Quantitatively, the percentage of tetraploid myocyte nuclei decreased in CAN rats by 48% (P<.03) in the left ventricle and 33% (P<.04) in the right ventricle (Fig 7). In contrast, diploid nuclei increased 8% (P<.05) and 6% (P<.004) in the left and right ventricles, respectively.

In summary, the DNA synthetic activity of myocyte nuclei after coronary artery narrowing was not coupled with the formation of polyploidy.

Identification of Mitosis in Myocytes

DNA synthesis in myocyte nuclei does not necessarily demonstrate that DNA replication was coupled with nuclear mitotic division. In addition, flow cytometric studies could not distinguish whether tetraploid nuclei represented cells in G2 or polyploid cells in G1. Therefore, after colchicine injection, light microscopic sections were examined to detect mitosis in myocytes at 1 and 2 weeks after surgery, since these intervals corresponded to high levels of BrdU labeling in the cells. This was important for the left ventricle because proliferation of myocyte nuclei was not demonstrated on this
TABLE 2. Effects of Coronary Artery Narrowing on the Distribution of Nuclei in Left and Right Ventricular Myocytes

<table>
<thead>
<tr>
<th></th>
<th>Time After CAN</th>
<th>% Change SO vs 1 Wk</th>
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<td>SO 1 Wk 2 Wk 1 Mo 3 Mo</td>
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<td>SO 1 Wk 2 Wk 1 Mo 3 Mo</td>
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**Left ventricle**

Myocyte nuclei

| % Nuclei in mononucleated cells | 2.2±0.2 1.5±0.1 | -31* | 1.8±0.3 1.7±0.3 | 1.8±0.3 | -19 16 0 2 |
| % Nuclei in binucleated cells   | 96.1±0.6 96.0±0.2 | 0   | 95.9±1.0 96.6±0.3 | 97±0.4 | 1 1 1 0 |
| % Nuclei in trinucleated cells  | 0.4±0.2 0.4±0.1 | -2  | 0.6±0.5 0.4±0.2 | 0.3±0.1 | -23 -22 -52 -28 |
| % Nuclei in tetranucleated cells| 1.3±0.5 2.1±0.2 | 60  | 1.7±0.6 1.3±0.2 | 1.0±0.2 | -26 -53 -43 -22 |

Myocytes

| % Mononucleated cells | 4.3±0.4 3.0±0.3 | -30* | 3.5±0.5 3.4±0.6 | 3.5±0.7 | -19 15 -1 1 |
| % Binucleated cells   | 94.8±0.5 95.7±0.3 | 1   | 95.3±0.9 95.7±0.6 | 95.8±0.7 | 1 0 1 0 |
| % Trinucleated cells  | 0.3±0.1 0.3±0.1 | -1  | 0.4±0.3 0.3±0.1 | 0.2±0.1 | -23 -23 -52 -28 |
| % Tetranucleated cells| 0.6±0.2 1.0±0.1 | 61  | 0.8±0.3 0.6±0.1 | 0.5±0.1 | -26 -54 -43 -23 |

**Right ventricle**

Myocyte nuclei

| % Nuclei in mononucleated cells | 3.1±0.5 3.0±0.2 | -2  | 2.8±0.6 3.5±0.4 | 4.7±0.3 | 51* 54* 66* 34* |
| % Nuclei in binucleated cells   | 95.6±0.7 95.9±0.2 | 0   | 95.8±0.9 95.2±0.6 | 94.2±0.4 | -1 -2 -2 -1 |
| % Nuclei in trinucleated cells  | 0.4±0.3 0.3±0.2 | -28 | 0.6±0.3 0.3±0.1 | 0.2±0.1 | -50 -30 -65 -18 |
| % Nuclei in tetranucleated cells| 0.9±0.4 0.8±0.1 | -9  | 0.8±0.4 1.0±0.3 | 0.9±0.2 | -5 5 17 -16 |

Myocytes

| % Mononucleated cells | 6.0±0.9 5.9±0.4 | -2  | 5.5±1.1 6.8±0.7 | 9.0±0.5 | 49* 52* 63* 32* |
| % Binucleated cells   | 93.3±0.9 93.5±0.3 | 0   | 93.7±1.3 92.5±0.8 | 90.5±0.6 | -3 -3 -3 -2 |
| % Trinucleated cells  | 0.3±0.2 0.2±0.1 | -28 | 0.4±0.2 0.2±0.1 | 0.1±0.1 | -51 -31 -66 -19 |
| % Tetranucleated cells| 0.4±0.2 0.4±0.1 | -9  | 0.4±0.2 0.5±0.1 | 0.4±0.1 | -6 3 5 -17 |

CAN indicates coronary artery narrowing; SO, sham-operated animals. Results are presented as mean±SD.

*Statistically significant, P<.05.

side of the heart (Fig 1). Fig 8 illustrates metaphase chromosomes in myocyte nuclei of the left ventricle 1 week after coronary narrowing (Fig 8A through 8D). Similar images were found in the right ventricle (data not shown). In addition, mitotic figures were seen at 2 weeks, 1 month (Fig 9A), and 3 months after surgery. It should be emphasized, however, that a quantification of this phenomenon was impossible. In most cases, it was extremely difficult to establish whether a mitotic image pertained to a myocyte or a nonmyocyte cell (Fig 9B). Dividing myocytes lose part of their characteristic appearance, making their recognition very complex.

In summary, myocyte nuclear mitotic division occurred in the left and right ventricles after coronary artery narrowing and persisted with time.

**Distribution of Nuclei in Myocytes**

The recognition that myocytes can synthesize DNA and undergo nuclear mitotic division does not demonstrate that cellular division occurs. The generation of new nuclei may be coupled with an increased number of nuclei per cell without affecting the number of myocytes. Therefore, the fraction of mononucleated, binucleated, and multinucleated myocytes was measured in SO and CAN rats at 1 week, 2 weeks, 1 month, and 3 months after surgery (Table 2). Since the proportion of the different populations of myocytes was almost identical in SO rats at the various time points, these animals were combined in a single group. Binucleated myocytes constituted 93% to 96% of the myocyte population of both ventricles, and this value did not change after coronary constriction. Mononucleated cells accounted for 5% to 7% of myocytes. Mononucleated cells did not change in the left ventricle with CAN but increased by 49% (P=.002) in the right ventricle at 3 months.

Knowledge of the distribution of nuclei in myocytes (Table 2) and the total number of myocyte nuclei in the ventricles (Fig 1) allowed the computation of the number of cells in the ventricles. The left ventricle possessed 0.96×10^6 mononucleated and 21×10^6 binucleated myocytes (Fig 10). Coronary constriction produced 44% (P=.0001) and 32% (P=.0001) losses in mononucleated and binucleated myocytes. Trinucleated cells decreased 49% (P=.005) and tetranucleated cells, 48% (P=NS). In the right ventricle (Fig 11), mononucleated myocytes increased 110% (P=.0001) 3 months after coronary stenosis. Moreover, binucleated myocytes increased 36% (P=.0001) and tetranucleated myocytes, 38% (P=.02). In contrast, trinucleated myocytes decreased by 31% (P=NS). Finally, the aggregate number of myocytes was decreased 32% (P=.0001) in the left.
Left Ventricle

![Bar graphs showing effects of coronary artery narrowing (CAN) of 3 months' duration on the total number of mononucleated, binucleated, trinucleated, and tetranucleated myocytes in the left ventricular myocardium. Results are presented as mean±SD. *Significantly different, P<.05. SO indicates sham-operated control animals. SO, n=12; CAN, n=13.](image)

The left ventricle, whereas the same parameter was increased 40% (P<.0001) in the right ventricle (Fig 12).

In summary, coronary artery constriction resulted in myocyte loss and myocyte cellular hyperplasia.

**Discussion**

The results of the present study demonstrate that the cellular mechanisms of ventricular remodeling in chronic ischemia involved myocyte loss, myocyte cellular hypertrophy, and myocyte cellular hyperplasia, which together contributed to the development of decompensated eccentric left ventricular hypertrophy. The magnitude of myocyte proliferation in the left ventricle was not sufficient to compensate for the loss of cells, and the consequent reduction in the number of myocytes appeared to be a critical factor in the persistence of ventricular failure and the progression of the disease toward its chronic irreversible phase. In contrast, the increase in aggregate number of myocytes in the right ventricle, in combination with limited myocardial damage, may account for the modest impairment of right ventricular performance in this setting. Although myocyte loss and proliferation affected the left and right ventricles in different proportions, in both cases, stimulation of interstitial cells exceeded the reaction of myocytes leading to myocardial fibrosis. However, the myocyte response preceded that of nonmyocytes, suggesting that loading abnormalities may exert their primary effect on the muscle compartment of the myocardium.

**DNA Synthesis in Myocytes and Coronary Artery Constriction**

The present results demonstrate that coronary narrowing was associated with an increase in the number of myocyte nuclei synthesizing DNA in both ventricles. During the early and intermediate stages of ischemic cardiomyopathy, this reaction involved more than 1% of left ventricular myocyte nuclei and nearly 0.6% of right ventricular myocyte nuclei. Since the left and right ventricles possess 43.8×10^6 and 7.2×10^6 myocyte nuclei, 438000 and 43200 nuclei were undergoing DNA synthesis in the left and right ventricles, respectively. Importantly, small fractions of myocyte nuclei, 0.2% in the left and 0.15% in the right ventricle, showed BrdU labeling in SO rats, implying that DNA replication was occurring in 87600 left and 10800 right ventricular myocyte nuclei under control conditions. Therefore, DNA synthesis is normally present in differentiated myocytes, and this process is potentiated by coronary constriction.

The observations in this investigation are in contrast with previous studies in which pressure overload hypertrophy has been characterized by the lack of [3H]thymidine incorporation in myocyte nuclei. However, recent results have indicated that the number of myocyte nuclei incorporating labeled thymidine is 8.0×10^5 at birth and 1.4×10^6 in the adult rat heart. Although the 1.4×10^6 value is slightly higher than that found in the present work at baseline, the possibility that increases in
Right Ventricle

**Fig 11.** Bar graphs showing effects of coronary artery narrowing (CAN) of 3 months' duration on the total number of mononucleated, binucleated, trinucleated, and tetranucleated myocytes in the right ventricular myocardium. Results are presented as mean±SD.

*Significantly different, P<.05. SO indicates sham-operated control animals. SO, n=12; CAN, n=27.

load may activate DNA replication in myocytes may have to be reexamined. Moreover, DNA synthesis and myocyte mitotic division have been demonstrated previously after pressure overload hypertrophy in the adult rat.\textsuperscript{16} Conversely, light microscopic autoradiographic detection of thymidine-labeled myocyte nuclei\textsuperscript{13-16,42} or BrdU immunofluorescence localization in nuclei\textsuperscript{23} does not discriminate whether the DNA synthetic activity is coupled with nuclear hyperplasia, ploidy formation, or DNA repair.

The analysis of ploidy levels in myocytes 3 months after coronary constriction indicated that myocyte growth was not accompanied by a change in the distribution of ploidy. DNA replication in this period was, at most, minimally involved in the polyploidization of ventricular muscle cells. However, it cannot be excluded that this phenomenon may be a significant component of myocyte adaptation late in the evolution of the myopathy. Ploidy formation is a well-established event of the decompensated hypertrophied heart in humans,\textsuperscript{43-46} in which it correlates closely with the increases in myocardial mass.\textsuperscript{45} Aging does not seem to affect this process,\textsuperscript{44} so myocytes may retain their capacity to synthesize DNA throughout life.

**Myocyte Nuclear Mitotic Division and Coronary Artery Constriction**

In this study, mitotic images in myocytes were encountered at various times after coronary stenosis. Mitotic figures were present in left and right ventricular myocytes, but a quantitative evaluation of this process could not be performed. Difficulty existed in establishing whether an individual image was associated with a myocyte nucleus, a fibroblast, or an endothelial cell. During mitosis, myocytes do not retain their characteristic morphological appearance. Colchicine administration increased the number of mitoses in both myocytes and noncontractile cells, but this alkaloid alters the configuration of metaphase chromosomes\textsuperscript{37} and did not improve the distinction between myocytes and nonmyocytes. The complexity of establishing the cell of origin in the presence of mitosis was indicated more than two decades ago,\textsuperscript{14} and the observations here confirm this contention. Moreover, only a few examples of mitosis in myocyte nuclei have been published in both the normal\textsuperscript{46} and hypertrophied\textsuperscript{16,23} rat heart. Such limitation in the recognition of mitosis may represent one of the most important reasons for the controversy on the existence of myocyte cellular hyperplasia in the mammalian heart.\textsuperscript{1,3} Conversely, it should be emphasized that mitotic images in myocyte nuclei alone cannot be interpreted as indicators of myocyte cellular hyperplasia. This phenomenon may simply reflect a change in the number of nuclei per cell without a real increase in myocyte number. However, DNA synthesis and nuclear mitotic division occur in adult ventricular myocytes. In view of these observations, the dogma that ventricular myocytes are terminally differentiated cells may be
challenged, first, on the basis of nuclear events and second, on the basis of the findings of myocyte proliferation discussed below. Thus, the possibility that myocyte hyperplasia represents a compensatory growth mechanism of the adult heart must be reconsidered.

**Myocyte Cellular Hyperplasia and Coronary Artery Constriction**

Results in this investigation indicate that the activation of DNA synthesis in myocytes and myocyte nuclear mitotic division were involved in the generation of new muscle cells after coronary artery stenosis. This contention was supported by the little changes in ploidy classes and in the distribution of nuclei in myocytes. In addition, the decrease in DNA synthesis in myocytes at 1 and 3 months after surgery suggests that the process of cellular hyperplasia was attenuated chronically. However, some proliferation of myocytes may persist because mitotic images in these cells were observed at these late intervals. Quantitative analysis of cell number at 3 months after coronary artery constriction demonstrated that the total number of myocytes in the right ventricle increased 40% as a result of 110% and 36% increases of mononucleated and binucleated myocytes, respectively. In contrast, myocyte proliferation in the left ventricle was not associated with an increase in the total number of myocytes. Myocardial damage and cell loss exceeded the hyperplastic response of myocytes so that the left ventricle possessed 44% and 32% fewer mononucleated and binucleated muscle cells. These losses resulted in a 32% reduction in the number of myocytes in the ventricle.

The data summarized above demonstrate that in 3 months, the right ventricle increased its number of cells by $1.49 \times 10^6$. Although the fraction of BrdU-labeled myocytes in the left ventricle was consistently higher than that in the right ventricle, it is impossible to establish with certainty the magnitude of cellular hyperplasia in this side of the heart. The presence of myocyte loss in this ventricle complicates the estimation of the amount of newly formed cells by any methodological procedure currently available. However, BrdU labeling of 43 200 nuclei in the right ventricle at 1 to 2 weeks after coronary stenosis was associated with the generation of $1.49 \times 10^6$ new myocytes at 3 months. If a similar relation existed in the left ventricle, the corresponding 438 000 BrdU-labeled nuclei should have resulted in the accumulation of $15.1 \times 10^6$ new myocytes. This would imply that coronary artery constriction was accompanied by a 69% increase in the number of left ventricular myocytes in 3 months. Conversely, cell loss involved 60% of the myocyte population, which accounted for $13.3 \times 10^6$ myocytes.

The present findings document not only that ventricular myocytes can proliferate but also that this process leads to the restoration of large quantities of muscle cells lost as a result of ischemic injury. This and previous observations are consistent with several reports in humans in which myocyte proliferation has been claimed in pathological conditions associated with increases in heart weight in excess of 500 g. The recognition that myocyte loss is a significant variable of cardiac disease processes, including myocardial aging, suggests that the critical heart weight theory may have to be reconsidered. Myocyte hyperplasia may be present more frequently than expected and masked by the phenomenon of cell loss. Moreover, as demonstrated here, myocyte mitotic division may occur concurrently with cellular hypertrophy and not as a secondary event that takes place after exhaustion of the hypertrophic growth capacity of these cells, as postulated in humans.

**Myocyte Cellular Hypertrophy and Coronary Artery Constriction**

Findings in this investigation indicate that myocyte cellular hypertrophy occurred in the left and right ventricles after coronary narrowing. In the left ventricle, a 49% enlargement of myocytes was observed 3 months after coronary stenosis. Myocyte cell volume in the right ventricle increased only 21%. A disproportion existed...
between the increases in left (12%) and right (68%) ventricular weights and the expansions in myocyte cell volume in the ventricles. This apparent inconsistency reflects the impact of myocyte loss, myocyte cellular hyperplasia, and myocyte cellular hypertrophy on the overall response of the myocardium to chronic ischemia. A similar dissociation between cardiac weight changes and cellular changes has been found with myocardial infarction, aging, and hypertension. These observations document the complexity of the cardiac hypertrophy process and the necessity of measuring myocyte size and number and tissue properties to identify the adaptive and maladaptive components of ventricular remodeling in pathological states.

Ventricular Remodeling and Myocyte Hypertrophy and Hyperplasia

Left ventricular failure after coronary artery constriction was associated with cavity dilation and a reduction in the thickness of the left ventricular wall. Conversely, right ventricular dysfunction was accompanied by an expansion in chamber volume that was comparable to the increase in mural thickness. Thus, these hemodynamic and anatomic alterations are consistent with eccentric hypertrophy in its uncompensated form. The phenomenon of maladaptation, however, was much more severe in the left than in the right ventricle. This differential response between the ventricles may find its basis in the inability of left ventricular myocytes to regenerate an adequate number of cells by mitotic division. Myocyte loss can be expected not only to result in an elevation in load on the existing myocytes but also to affect ventricular dimension acutely and chronically. The acute consequences of cell loss reflect the necrotic phase in which dying myocytes are overstretched in diastole and noncontracting in systole, resulting in diastolic and systolic wall thinning. The chronic effects include the reparative processes associated with scar formation, which also reduce mural thickness and increase cavity diameter.

The possibility that ongoing myocytolytic necrosis plays a central role in the genesis of chamber enlargement with coronary artery stenosis has been raised previously. In addition, myocyte lengthening has been shown to be a component of the hypertrophic response of left ventricular myocytes, contributing to the expansion in cavity volume. Whether structural rearrangement of myocytes within the wall involving side-to-side slippage of cells participates in the dilation of the chamber and the development of uncompensated eccentric hypertrophy cannot be established here. Similarly, the present results cannot determine whether the phenomenon of myocyte cellular hyperplasia was coupled with the in-series addition of newly formed cells, resulting in an increase in the ventricular chamber volume.

The morphometric approach used for the evaluation of the number of myocytes across the right ventricular free wall, their mean center-to-center distance, and their average cross-sectional diameter has allowed the interpretation of the gross anatomic parameters of right ventricular dimension at the cellular level. The 31% increase in wall thickness associated with coronary artery narrowing was the result of a 10% increase in myocyte diameter and a 20% increase in the number of myocytes within the ventricular wall. However, the total number of right ventricular myocytes increased by 40%, which was twofold greater than the increase in the mural number of cells. Thus, myocyte proliferation was associated with the parallel and in-series addition of newly formed cells in the myocardium. This latter form of myocyte growth accounted for the expansion in cavity volume, documented by the 28% increase in right ventricular wall area.

The pattern of myocyte growth summarized above is consistent with the increase in right ventricular peak systolic and end-diastolic pressures found in the present study. The adaptations of myocytes may be interpreted as a compensatory response of the myocardium at the cellular level that tends to minimize the effects of an increased load on the heart. According to the law of Laplace, the larger myocyte diameter and the greater number of cells across the wall would produce a proportional thickening of the wall that should offset the higher peak systolic wall stress resulting from the elevation in pressure. Conversely, the longitudinal insertion of new myocytes would counteract the greater end-diastolic wall stress by contributing to the enlargement in chamber volume. In essence, the mechanism of cellular hyperplasia demonstrated here, in which new myocytes were added laterally within the wall and in series in the myocardium, had the beneficial effect of decreasing systolic and diastolic stresses on a per-cell basis, improving the myocardial response to pressure and volume overload hypertrophy.

Acknowledgments

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Myocyte cellular hyperplasia and myocyte cellular hypertrophy contribute to chronic ventricular remodeling in coronary artery narrowing-induced cardiomyopathy in rats.

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