Morphometry of Right Ventricular Hypertrophy Induced by Myocardial Infarction in the Rat

PIERO ANVERSA, MD, CESARE BEGHI, MD, SUSAN L. McDONALD, BS, VLADIMIR LEVICKY, MD, YUTAKA KIKKAWA, MD, and GIORGIO OLIVETTI, MD

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

The growth response of the right ventricle was studied in rats following ligation of the left coronary artery, which produced infarcts comprising approximately 40% of the left ventricle. A month after surgery the weight of the right ventricle was increased 30%, and this hypertrophic change was characterized by a 17% wall thickening, consistent with the 13% greater diameter of myocytes. Myocardial hypertrophy was accompanied by an inadequate growth of the microvasculature that supports tissue oxygenation. This was seen by relative decreases in capillary luminal volume density (−27%) and capillary luminal surface density (−21%) and by an increase in the average maximum distance from the capillary wall to the mitochondria of myocytes (19%). In contrast, measurements of the mean myocyte volume per nucleus showed a proportional enlargement of these cells (32%), from 16,300 cu μ in control animals to 21,500 cu μ in experimental rats. Quantitative analysis of the right coronary artery revealed a 33% increase in its luminal area, commensurate with the magnitude of ventricular hypertrophy. (Am J Pathol 1984, 116:504–513)

EXPERIMENTAL studies have demonstrated that after large infarcts of the left ventricle in rats, produced by ligation of the left coronary artery in the region of its origin, there is a relatively rapid development of right ventricular hypertrophy.[1-3] The hypertrophic growth response of the right ventricle was observed as early as 21 days after coronary occlusion[3] and was still present 12 weeks after the operation.[1] These observations indicate that hypertrophy of the right ventricle develops during the healing process, usually complete by Day 21,[4] and persists later in life as a specific characteristic of the myocardial changes involved in the adaptation of the heart following extensive infarcts of the left ventricle.

The precise mechanism by which myocardial infarction leads to right ventricular hypertrophy is currently unknown. Three possibilities have been considered: 1) There might be an increase in right ventricular systolic pressure that would have the tendency to maintain the pressure gradient across the pulmonary bed in left-side pump failure.[3] 2) Pulmonary hypertension might be a primary stimulus, suggested by medial hypertrophy of the muscular branches of the pulmonary artery in infarcted rats.[2] 3) The right ventricle may constitute a functional unit with the infarcted left ventricle, contributing to the emptying of the left ventricular chamber during systole.[2,5] These events, either independently or combined, should lead to a greater pressure load of the right ventricle, resulting in concentric hypertrophy.

The present study was designed to evaluate the ability of right ventricular myocardium to respond to the stress of extensive infarcts of the left ventricle. The compensatory growth of the right ventricle was analyzed morphometrically by measuring the structural parameters of the capillary bed controlling tissue oxygen distribution and consumption; the size, shape, and number of myocytes; and the subcellular components of myocytes responsible for energy production and utilization. Acquired right ventricular hypertrophy implies an increase in muscle mass in which total coronary blood flow should increase in order to maintain an adequate perfusion per gram of

Supported by US PHS Research Grants HL-24479 and HL-24817 from the Heart, Lung and Blood Institute, National Institutes of Health, and by a Grant from the Consiglio Nazionale delle Ricerche (CNR).

Accepted for publication April 27, 1984.

Address reprint requests to Dr. Piero Anversa, Department of Pathology, New York Medical College, Valhalla, NY 10595.

muscle. This compensation can be achieved on a structural basis by either vasodilatation of the right coronary artery or by proliferation of capillary units, or both. Therefore, the estimation of the tissue properties of the myocardium at the capillary level was combined with the measurement of the changes in size of the major right coronary artery.

Materials and Methods

Ligation of the left coronary artery was performed in 40 male Wistar–Kyoto rats at 12 weeks of age (Charles River, Breeding Labs, North Wilmington, Mass) using a technique described in detail elsewhere.[6] Briefly, with the rats under ether anesthesia, the thorax was opened by an incision to the left of and parallel to the sternum, and the heart was exteriorized by applying light pressure upon the thorax. The left coronary artery was then ligated 1–2 mm from its origin for production of an infarct that comprises approximately 40% of the volume of the left ventricle.[4] The chest was closed, and the animals were allowed to recover. Twenty-nine rats died shortly after the operation, mostly because of pulmonary edema due to excessive infarct size incompatible with survival. The remaining 11 animals were sacrificed 30 days later. An additional group of 10 animals in which a sham operation was performed by placing an incomplete ligature around the coronary artery was used as controls and similarly sacrificed a month after surgery.

Just prior to sacrifice all animals were anesthetized with Nembutal (sodium pentobarbital, Abbott Laboratories, North Chicago, Ill; 3 mg/100 g body weight, intraperitoneally) and after isolating the abdominal aorta a polyethylene cannula filled with pH 7.2 phosphate buffer containing 100 IU heparin/ml was inserted into the aorta and sealed in place with a ligature. The cannula was attached to a perfusion apparatus. In rapid succession, the heart was arrested in diastole by injection of approximately 1 ml of 1 M KCl through the jugular vein, the thorax opened, perfusion with buffer started, and the right atrium cut to allow the drainage of blood and perfusate. Perfusion pressure was adjusted to 85 mm Hg, approximately equal to the average diastolic pressure in adult anesthetized rats.[7] After perfusion with buffer for 3 minutes, the coronary vasculature was perfused for 15 minutes with a glutaraldehyde–paraformaldehyde mixture diluted 1:1 with phosphate buffer.

After fixation *in situ*, the heart was excised; and under a dissecting microscope, the atria and pulmonary artery were trimmed off. The origin of the right coronary artery was located by opening longitudinally the posterior aspect of the aorta near the cusps of the aortic semilunar valve, and approximately 2 mm of the axial length of the right coronary artery were dissected free. Subsequently, the free wall of the right ventricle was dissected and weighed, and the tissue weight of the left ventricle, including the septum, was determined. The free wall of the right ventricle was then sliced transversely into several thin arcs, from which 30 blocks extending from the epicardial to the endocardial surface were obtained. Finally, the whole left ventricle and its chamber were supported with agar (5% agar in distilled water) and serially sectioned into 1-mm-thick rings perpendicular to the axis of the heart from the apex to the base. The individually numbered slices of the left ventricle, including the septum, the 30 pooled blocks sampled from the right ventricle, and the small segment of right coronary artery obtained in each animal were fixed an additional 3–4 hours in fresh fixative, washed several times, and stored overnight in phosphate buffer, postfixed in 1% osmium tetroxide, dehydrated with acetone, and embedded in Araldite with large flat molds. At the time of embedding the segment of right coronary artery was cut perpendicular to the long axis and through the full thickness of the vessel for the purpose of obtaining four rings, approximately 0.5 mm in thickness, which were embedded with the distal end facing the surface of the flat molds. Similarly, the serial sections of the entire left ventricle were embedded with the basal side exposed to the surface of the molds.

Determination of Infarct Size

For evaluation of infarct size the mean thickness of each embedded serial section of each left ventricle was determined before sectioning by averaging five individual measurements made at a magnification of ×28 with a dissecting microscope having an ocular micrometer accurate to 0.03 mm. Sections from the basal side of each serial slice were cut at a thickness of 2.0–2.5 μ with a glass knife, 38 mm in length (LKB 2078 Histo Knife Maker), and the Sorvall JB-4 microtome (DuPont Company, Newtown, Conn) and mounted on glass slides. These sections, representing 12–14 uniformly spaced parallel planes through the whole left ventricle were stained with toluidine blue (1 percent aqueous solution) and used for measurement of the total length of endocardial circumference present in each section and the length of endocardial circumference delimiting the infarcted portion of the ventricle. These values were obtained by projecting each section on the digitizer tablet of a Videoplan image analysis system (Carl Zeiss, Inc., New York,

NY) and then circumscribing with the connected stylus the individual images (final magnification ×18). The two length measurements multiplied by the previously determined thickness of the embedded tissue slice yield the total endocardial area and the area of endocardium facing the infarcted myocardium in each slice. The summation of each set of data derived in each serial section of the ventricle gives the overall endocardial surface of the ventricular chamber and the amount of endocardial area reflecting the infarcted portion of the whole ventricle. Infarct size was calculated by dividing the latter value by the former to provide an estimate of the fraction of the left ventricle occupied by scarred tissue. This method is similar to that originally described by Fishbein et al,[4] and it has been repeatedly used in several laboratories.[3]

Determination of Right Ventricular Wall Thickness, Area, and Volume

Five tissue blocks were chosen at random from the 30 blocks of each right ventricular free wall; and sections, 0.5 μ thick, that included the entire wall thickness were cut and stained with toluidine blue. Each section was then projected on the digitizer tablet of the Videoplan (final magnification ×85), and the tracing of the wall area divided by the mean length of the projected image provides a direct estimate of mean wall thickness in each tissue section. These results were corrected for compression effects as previously described.[8] With a tissue density of 1.06,[9] the volume of the right ventricular free wall was calculated from its measured weight; and the mean area was obtained by dividing wall volume by wall thickness. In this calculation we assume that the ventricular wall may be treated as a thin sheet.

Determination of the Average Cell Volume per Nucleus of Right Ventricular Myocytes

Six to eight tissue blocks from each right ventricle were sectioned for light-microscopic nuclear counting at a nominal thickness of 0.75 μ with an MT-1 Porter-Blum microtome (Ivan Sorvall, Inc., Norwalk, Conn). The sections were stained with methylene blue and safranin and mounted on glass slides.[8] Morphometric sampling at a magnification of ×1000 consisted of counting the total number of myocyte nuclear profiles, N(n), in a measured area, A, in transversely oriented tissue sections. A total of 24 such fields were evaluated in each animal, 12 from the subendocardial region and 12 from the subepicardial region. A square tissue area equal to 8100 sq μ was delineated in the microscopic field by an ocular reticle (#105844, Wild Heerbrugg Instruments, Inc., Farmingdale, NY), and laterally adjacent fields were examined in each section. Nuclear counts were collected by the rules described by Gundersen,[10] and the data were corrected for the effect of section compression occurring during microtomy.[8]

Average nuclear length was determined in each animal from 95 to 105 measurements made at a magnification of ×1200 in longitudinally oriented myocytes located in the midregion of the right ventricular wall and viewed with a microscope having an ocular micrometer accurate to 0.001 mm. Eight to ten blocks were cut in each animal, and the longitudinally assembled myofibers were sectioned perpendicular to their length to avoid longitudinal compression. Sections, approximately 2.0 μ in thickness, were collected and stained, and 10 to 13 measurements of nuclear length were recorded from each tissue section. Only nuclei in which the nuclear envelope was sharply defined at both ends and also clusters of mitochondria were clearly visible in the areas adjacent to the nuclear edges were considered.

Measurements of the number and volume of myocytes in the right ventricular myocardium were obtained by simplifying a recently described morphometric technique.[8,11-13] The previously used stereologic method was based on the estimation of the number of myocyte nuclei per unit volume of myocardium, $N(n)_V$, derived from the average counts of nuclei per unit area, $N(n)_A$, of sections of known different thickness, t, using the equation:

$$N(n)_A = N(n)_V t + N(n)_V (\bar{D}_n - 2p)$$

The constant $(\bar{D}_n - 2p)$ is a measure of the mean nuclear dimension, $\bar{D}_n$, perpendicular to the plane of sectioning, minus twice the minimal thickness, p, of a nuclear profile that must be included within a slice in order for the nucleus to be visible. This linear relationship was derived from the general equation:

$$N(n)_V = N(n)_A / (\bar{D}_n - 2p + t)$$

described by several authors.[11]

We have shown previously that myocardial sections cut with glass knives and a Porter-Blum microtome at an accurately controlled nominal thickness of 0.75 μ were in actual thickness 0.96 ± 0.02 μ.[11] The uncompressed section thickness value, 0.92 μ, could then be obtained by multiplying the measured thickness by the compression factor, Fc = 0.957, subsequently evaluated.[8] Moreover, values for p have been found to be approximately 0.45 μ,[8] indicating that under these conditions −2p and +t are essentially compensating

each other. The above equation can, therefore, be written in a simpler form:

$$N(n)_V = N(n)_A/\bar{D}_n$$

and $N(n)_V$ calculated from the primary data of $N(n)_A$ and $\bar{D}_n$. The mean cell volume per nucleus, $\bar{V}(c)_n$, was then derived from

$$\bar{V}(c)_n = V(c)_V/N(n)_V$$

where $V(c)_V$ is the volume fraction of myocardium occupied by myocytes measured in low-power electron micrographs (see below).

Low-Power Electron-Microscopic Morphometry

Transverse myocardial sections of two blocks from each right ventricle, one from the endocardial region and one from the epicardial region, were used for morphometric sampling by electron microscopy. Twelve random micrographs were taken at ×1800 and printed at ×4400, as determined by a diffraction grating replica magnification standard (E. F. Fullam, Inc., Schenectady, NY). A 194 × 247-mm grid containing 130 sampling points and 2522 mm of sampling line was superimposed on each print. The tissue area represented by each point was 19.04, sq μ, which, when corrected for the effect of section compression (Fc = 0.905,[8]), yields an area per point of 21.04 sq μ. The volume fractions of myocytes, myocyte nuclei, capillary lumen, capillary endothelium, and other interstitial structures were estimated by the fraction of sampling points overlying each of these components. Measurements of the surface areas of cell membranes, determined from the frequency of profile intersections with the sampling line, included the luminal surface of capillary endothelial cells and the sarcolemma and intercalated disk of myocytes. The numbers of myocyte profiles and capillary profiles per unit area of tissue cross-section were also counted, according to the criteria described by Gundersen.[10] The number of profiles of myocytes and capillaries were counted for measurement of their numerical density, average cross-sectional area, and length per unit volume. Standard morphometric relationships and compression correction factors[8,12] were used for volume, surface, and number measurements.

The maximum diffusion distance for oxygen, represented by the maximum distance from the capillary wall to the mitochondria of myocytes, R, was calculated from the capillary profile density in transverse myocardial sections[14,15] according to the equation:

$$R = \sqrt{\frac{1}{N(cap)_A\,\pi}} - \sqrt{\frac{\bar{A}cap}{\pi}}$$

where $N(cap)_A$ corresponds to the number of capillaries per unit area of myocardium and $\bar{A}cap$ is the average cross-sectional area of capillary lumen. This measurement, based on the Krogh's cylinder model for gas exchange in tissue,[14] assumes that capillaries are uniformly distributed in the myocardium and that the mitochondria are dispersed evenly in the myocyte cytoplasm.

High-Power Electron-Microscopic Morphometry

The overall composition of cytoplasm in the myocytes of the right ventricle was determined from 8 micrograph prints from each animal at a magnification of ×40,000. Measurements were made of the volume fractions of mitochondria, myofibrils, and matrix. The matrix compartment included glycogen, ribosomes, lipid, Golgi apparatus, smooth endoplasmic reticulum, rough endoplasmic reticulum, and amorphous regions. These volume determinations were obtained with the use of the 130-point morphometric grid.

Morphometry of Right Coronary Artery

Sections 0.5 μ thick, which included the entire circumference of each coronary ring, were cut from the four blocks collected from each animal, stained with toluidine blue, and examined with a microscope having an ocular micrometer accurate to 0.01 mm. Maximal and minimal internal diameters of each ring were measured at a magnification of ×125. Micrometric estimations of the thickness of the vessel wall were made at ×500 on each section along radii distributed 0, 90, 180, and 270 degrees around the circumference of each ring. The geometric mean value of luminal diameter was then calculated, and this measurement, as well as the wall thickness average value, was corrected for the effect of compression artifact by dividing the primary data in each animal by $\sqrt{Fc}$.[16] The cross-sectional areas of the lumen and wall were calculated from these corrected dimensions; we assumed they had a circular shape.

All of the results in each table show the mean values (± SD) computed from the average measurements obtained from each rat. Statistical significance was evaluated by the Student *t* test, and *P* values of less than 0.05 were considered to be significant.

Results

Table 1 shows that ligation of the left coronary artery near its origin produced large infarcts comprising 40% of the left ventricle, including the septum. Average heart weight in the experimental group was in-

Table 1—Gross Cardiac Changes Following Myocardial Infarction

	Control	Experimental	% Difference	$P <$
Number of rats	10	11		
Body weight (g)				
Surgery	253 ± 31	263 ± 19	4	NS
Sacrifice	321 ± 28	316 ± 19	−2	NS
Heart weight (mg)	1119 ± 96	1230 ± 115	10	0.05
Right ventricle	248 ± 32	322 ± 51	30	0.005
Left ventricle	871 ± 68	908 ± 117	4	NS
Right ventricular wall thickness (μ)	903 ± 115	1057 ± 145	17	0.002
Mean area (sq mm) of right ventricular free wall	259 ± 42	288 ± 49	11	NS
Infarct size (% of left ventricle)		40.2 ± 6.1		

Results are expressed as the mean ± SD.

creased 10%, and the change of 111 mg was mostly contained in a 30% increase in right ventricular weight (74 mg). Table 1 also demonstrates that the mean thickness of the free wall of the right ventricle was 17% greater in animals with infarcts, whereas the 11% increment in wall area value was not significantly different.

Low-power electron-microscopic analysis of the volume fractions of individual tissue components in normal and hypertrophied right ventricles are listed in Table 2. These figures show that the volume percent of capillary lumen was altered as a result of hypertrophy. The measured 27% reduction in capillary luminal volume was compensated for by a 31% increase in the other interstitial constituents of the ventricle. In contrast, the relative volume of myocytes remained essentially constant.

Table 3 demonstrates that the number of capillary profiles per unit area of myocardium was markedly decreased (−19%) in the enlarged ventricles. Similarly, capillary luminal surface area per unit volume of myocytes was significantly reduced (−21%) in rats with infarcts. The number of capillary profiles per square millimeter of myocardium was compared with the corresponding frequency of myocyte profiles, and the calculated ratios showed practically no variation, with values of 0.83 and 0.85 in control and experimental animals, respectively. Finally, the average maximum distance from the capillary wall to the mitochondria of myocytes was measured and found to be 19% greater in hypertrophy.

Table 4 shows the area of myocytes sampled and the number of nuclei collected from the light-microscopic cross-sections of right ventricular myocardium. In this sampling, in both groups of animals, a total of 3138 myocyte nuclei has been counted in 504 microscopic fields. The number of myocyte nuclei per square millimeter cross-sectional area of myocytes was then derived in each group of rats and found to be 18% less in animals with infarcts. In contrast, average nuclear length, obtained from a total of 2103 measurements made in longitudinally oriented myocytes, was significantly increased (9%) in the enlarged ventricles.

The number of nuclei per 10^6 cubic microns of myocytes is shown last in Table 4, and the mean cell volume per nucleus estimated from this measurement is listed at the top of Table 5. The mean cellular volume per nucleus is strictly equivalent to the mean cellular volume only in mononucleate cell populations. Since binucleate myocytes do exist in the right and left ventricular myocardium of Wistar Kyoto rats,[17] and their relative frequency cannot be consistently assessed by *in situ* analysis of histologic tissue sec-

Table 2—Volume Composition of Right Ventricular Myocardium

	Control	Experimental	% Difference	$P <$
Volume percent of myocardium				
Myocytes	82.47 ± 2.36	82.27 ± 2.84	0	NS
Nucleus	1.25 ± 0.31	1.12 ± 0.36	−10	NS
Cytoplasm	98.75 ± 0.31	98.88 ± 0.36	0	NS
Capillaries	10.22 ± 2.16	8.18 ± 1.84	−20	0.05
Lumen	7.98 ± 2.00	5.81 ± 1.52	−27	0.02
Endothelium	2.24 ± 0.22	2.37 ± 0.79	6	NS
Other interstitial structures	7.31 ± 1.67	9.55 ± 2.79	31	0.05

Results are expressed as the mean ± SD.

Table 3—Characteristics of Capillaries in Right Ventricular Myocardium

	Control	Experimental	% Difference	P<
Transverse luminal area of capillary profiles (sq μ)	27.97 ± 5.28	25.63 ± 8.58	−8	NS
Number of capillary profiles per square millimeter of myocardium	2927 ± 444	2360 ± 563	−19	0.05
Number of myocyte profiles per square millimeter of myocardium	3537 ± 505	2771 ± 376	−22	0.005
Ratio of capillary profiles to myocyte profiles	0.830 ± 0.085	0.853 ± 0.170	3	NS
Capillary luminal surface (sq mm) per cubic millimeter of myocytes	62.92 ± 8.95	49.48 ± 9.34	−21	0.005
Maximum diffusion distance for oxygen (μ)	7.56 ± 0.65	9.03 ± 1.31	19	0.01

Results are expressed as the mean ± SD.

tions,[8,12,13] the more general concept of mean cell volume per nucleus was again introduced here following the rationale previously discussed in detail.[8,13]

Table 5 presents the dimensional characteristics of ventricular myocytes. The 24% decrease in the concentration of nuclei per unit volume of myocytes in the experimental group (Table 4) produced a 32% enlargement of the mean cell volume per nucleus, from 16,300 to 21,500 cu μ. The average transverse cross-sectional area of these cells was 28% greater in hypertrophy, and this change corresponds to a 13% increase in mean cross-sectional diameter. The decrease in surface to volume ratio of myocytes (−13%) is consistent with the increase in size of these cells.

Analysis of organelle changes in myocytes is presented in Table 6. No significant alterations in the volume densities of myofibrils, mitochondria, and matrix were seen, although there was a tendency for a decrease in the mitochondrial fraction and an increase in the myofibrillar compartment of the cytoplasm.

To obtain an overall view of the growth of the myocardium, the total volume change of the right ventricular free wall is shown at the top of Table 7 and is combined with the preceding morphometric data to summarize the overall hypertrophy of the myocytes, capillaries, and other interstitium during the 1-month period following myocardial infarction. Volume gains of myocytes, capillary endothelium, and interstitium were 29%, 39% and 69%. These changes indicate that the muscle mass and the endothelium have expanded almost in proportion to the enlargement of the ventricle as a whole (30%), whereas a disproportionate growth adaption has occurred in the interstitium exclusive of capillaries. The presence of no change in total myocyte length and in the number of myocyte nuclei demonstrates that the contractile mass has increased only by lateral expansion of the existing myocytes without either hyperplasia or loss of these cells.

Table 8 shows that after surgical constriction of the left coronary artery the mean luminal diameter of the right coronary artery, within the first 2 mm from its origin, significantly increased by 16%. Since the ratios of maximal to minimal diameter of the coronary arteries for the control and experimental animals were sufficiently close to unity (control = 1.15; experimental = 1.21), computations of the cross-sectional areas of the lumen and wall were based on a circular shape. The transverse areas of the lumen and wall were increased 33% and 54%, producing an overall 38% increment in the area of the whole coronary artery. Although the standard deviations of these measurements were found to be even greater than 40% of the mean values, the results obtained indicate a more than adequate compensatory growth of the right coronary artery in ventricular hypertrophy.

Discussion

The results of the present study indicate that ligation of the left coronary artery near its origin in

Table 4—Numerical Density of Myocyte Nuclei in Right Ventricular Myocardium

	Control	Experimental	% Difference	P<
A	1.677	1.818		
Nn	1,659	1,479		
$N(n)_A$	990 ± 77	814 ± 127	−18	0.005
$\bar{D}n$	16.18 ± 0.79	17.57 ± 1.23	9	0.01
$N(n)_V$	61.22 ± 4.13	46.59 ± 8.11	−24	0.0001

Results are expressed as the mean ± SD.

A, total area of myocytes sampled (sq mm); Nn, total number of myocyte nuclear profiles counted in the sampled area; $N(n)_A$, number of myocyte nuclei per square millimeter of myocytes; $\bar{D}n$, longitudinal diameter of myocyte nuclei (length = μ) derived from a total of 978 and 1125 measurements made in control and experimental animals, respectively; $N(n)_V$, number of myocyte nuclei per 10^6 cu μ of myocytes.

Table 5—Dimensional Characteristics of Right Ventricular Myocytes

	Control	Experimental	% Difference	P<
Myocyte cell volume per nucleus (cu μ)	16,300 ± 1120	21,500 ± 4200	32	0.005
Transverse cross-sectional area of myocytes (sq μ)	237 ± 28	303 ± 48	28	0.005
Myocyte length (μ) per nucleus	68.8 ± 11.2	71.0 ± 14.6	3	NS
Myocyte surface (sq mm) per cubic millimeter of myocytes	277 ± 27	240 ± 21	−13	0.005

Results are expressed as the mean ± SD.

Wistar Kyoto rats produces large infarcts of the left ventricle estimated to comprise 40% of the wall after the healing process is completed, approximately a month following coronary occlusion.[4] The weight of the free wall of the right ventricle was found to be increased by 30%, demonstrating that a significant amount of right ventricular hypertrophy develops when there is a major loss of myocardial tissue in the left ventricle. This adaptation of the heart was observed previously in several studies with the same animal model, in which the hypertrophic growth of the right ventricle was explained on the basis of an increased pressure load on the ventricle.[2,3,5] Increasing pressure load in the adult heart induces concentric hypertrophy, in which wall thickness increases without chamber enlargement.[18] Concentric hypertrophy of the right ventricular free wall is demonstrated in the present investigation by its 30% overall growth, 17% wall thickening, and 11% expansion in mean wall area. The changes in wall thickness and area, in fact, are consistent with the 13% greater average transverse diameter of myocytes. In addition, total aggregate length of myocytes was found to be identical in control and experimental rats, further confirming the lack of chamber dilatation in the hypertrophied ventricles.

As a result of tissue hypertrophy in the right ventricle, capillary luminal volume percent decreased from 7.98 in controls to 5.81 in experimental animals. Absolute capillary volume, however, remained practically constant. These structural parameters, which are related to the amount of blood available for gas exchange within the tissue,[14,15] indicate a local reduction of capillary blood in hypertrophy. In contrast, capillary luminal volume fraction in the left ventricular myocardium is not altered following pressure overload hypertrophy in either the inner, middle, or outer region of the ventricular wall or in the papillary muscle.[8,12,19] The different response capacities of the two ventricles is difficult to explain. Since the structural properties of both populations of capillaries are alike[20] and the degree of hypertrophy does not seem to be a significant factor,[21] the inadequate growth adaptation of the capillary bed in the right ventricle could be mostly related to the different nature of the inciting stimulus.

The volume fraction of capillary lumen in the myocardium is the product of the number of capillary profiles per square millimeter of tissue and the mean luminal cross-sectional area. In hypertrophy capillary density decreased (−19%) as the individual myocytes increased in diameter (13%). The average size of capillaries was also reduced (−8%), although this alteration was not statistically significant. Changes in capillary size and number imply corresponding changes in the luminal surface area of capillaries, the area available for oxygen transport.[14,15] Capillary luminal surface area per unit volume of myocytes was found to be decreased by 21%, slightly more than capillary density because of the concomitant decrease in capillary diameter. A further compromise of the structural properties relevant to tissue oxygenation was seen in the increased maximum diffusion distance from the capillary wall to the mitochondria of myo-

Table 6—Volume Composition of Myocyte Cytoplasm

	Control	Experimental	% Difference	P<
Volume percent of cytoplasm				
Myofibrils	49.87 ± 3.86	52.77 ± 4.48	6	NS
Mitochondria	33.63 ± 3.09	31.32 ± 4.11	−7	NS
Matrix	16.50 ± 3.19	15.91 ± 1.91	−4	NS
Mitochondrial volume/myofibrillar volume	0.681 ± 0.103	0.603 ± 0.127	−11	NS

Results are expressed as the mean ± SD.

Table 7—Changes in Right Ventricular Myocardium: Absolute Component Volumes, Lengths, Surface Area, and Number

	Control	Experimental	% Difference	P<
Volume (cu mm)				
Ventricular wall	234 ± 30	304 ± 48	30	0.005
Myocytes	194 ± 28	251 ± 43	29	0.005
Capillary lumen	18.48 ± 3.97	17.69 ± 5.39	−4	NS
Endothelium	5.24 ± 0.63	7.28 ± 2.69	39	0.05
Other interstitium	16.89 ± 4.44	28.50 ± 6.95	69	0.001
Length (m)				
Myocytes	834 ± 180	844 ± 186	1	NS
Capillaries	689 ± 157	732 ± 264	6	NS
Surface area (sq mm)				
Capillaries	12,200 ± 2600	12,400 ± 3100	2	NS
Number of myocyte nuclei $\times 10^{-6}$	11.79 ± 1.41	11.48 ± 1.77	−3	NS

Results are expressed as the mean ± SD.

cytes (19%), demonstrating a greater path length for molecular oxygen transport within the tissue. Pressure overload hypertrophy of the left ventricle, on the other hand, typically results in an increase in capillary luminal cross-sectional area, maintenance of capillary luminal surface density of the myocardium,[8,12,19] and no change in the average diffusion distance for oxygen.[21] Since focal areas of necrosis and fibrosis throughout the ventricular wall were not observed in the present study, it remains to be determined how much capillary lumen volume and surface must decrease and the diffusion distance for oxygen increase before effective hypoxia develops in the myocardium.

The process of capillary proliferation does not occur in pressure overload hypertrophy of adult myocardium. This is evident from previous observations on the left ventricle[8,19] and is confirmed here in the right ventricle on the basis of a lack of significant changes in capillary density, capillary-to-myocyte ratio, and in the total length of capillaries in the whole ventricle. On the other hand, volume overload hypertrophy obtained by subjecting adult rats to an exercise regimen is associated with capillary proliferation[22–25] that leads to the preservation of a larger quantity of viable tissue after coronary artery ligation.[24]

When faced with a sustained increase in workload, the adult myocardium responds with generally characteristic changes at the cellular level of organization. Increased cardiac mass is the result of the development of larger myocytes,[17,19,26] whereas cellular hyperplasia and cellular hypertrophy both contribute significantly to the expansion of the interstitial cell population.[8] The present data indicate that the growth of the contractile mass measured by volume was 29%, and independent measurements of the mean myocyte volume per nucleus showed an average cellular hypertrophy of 32%, from 16,300 cu μ in control animals to 21,500 cu μ in experimental rats, consistent with the the view that myocytes do not exhibit cellular hyperplasia during cardiac hypertrophy. This is confirmed further by the practically similar number of myocyte nuclei in the ventricles of the two groups of animals.

The method employed here for estimation of the numerical density of myocyte nuclei and the mean cell volume per nucleus was developed so that we might take advantage of the oriented distribution of

Table 8—Changes in the Right Coronary Artery Following Myocardial Infarction

	Control	Experimental	% Difference	P<
Right coronary artery				
Luminal diameter (μ)	263 ± 37	304 ± 43	16	0.05
Wall thickness (μ)	18.10 ± 6.09	24.32 ± 7.27	34	NS
Cross-sectional area (sq μ)				
Coronary artery	71,700 ± 21,500	99,000 ± 25,500	38	0.025
Lumen	55,300 ± 15,800	73,700 ± 19,700	33	0.05
Wall	16,400 ± 7200	25,300 ± 9400	54	0.05

Results are expressed as the mean ± SD.

muscle fibers in the myocardium. It is noteworthy to indicate that the mean cell volume per nucleus recently measured from isolated myocytes of the right ventricle of Wistar Kyoto rats[17] is nearly the same as that found in the present study. Slightly smaller values were obtained in a previous investigation performed in our laboratory,[11] but there correction for compression artifact during microtomy[8] and the counting rules for object images pointed out by Gundersen[10] were not applied. Both these factors would have the effect of decreasing the numerical density of nuclei, thus increasing the mean cell volume per nucleus.

The morphometric evaluation of subcellular alterations in hyperptrophied myocardium indicates that cellular enlargement decreased the relative surface area of the external sarcolemma (−13%) because of the fact that the myocytes assume a generally broader configuration. However, it has been repeatedly shown that the surface area of the sarcolemma constituting the transverse tubular system expands significantly more than the mean cellular growth to maintain a constant cell surface/volume ratio in hypertrophy.[21,27]

The mitochondria represent the primary source of energy for myofibrils through the generation of adenosine triphosphate in the mitochondrial cristae.[28] Although the estimation of the surface density of mitochondrial inner membranes per unit volume of mitochondria would provide the most significant structural parameter directly related to the oxidative capacity of these organelles, this morphometric measurement is difficult to obtain and is affected by several systematic methodological errors.[29] Furthermore, it has been demonstrated that the concentration of mitochondrial cristae in both cardiac and skeletal muscle fibers remain practically constant under a variety of stresses.[8,27,29] Thus, the quantitative evaluation of the volume fraction of mitochondria in the cell cytoplasm is considered a reliable index of the oxidative capacity of muscle tissue.[29] Data in the present study indicate a 7% reduction in the mitochondrial volume density and an 11% decrease in the mitochondria/myofibril volume ratio, but neither of these alterations were statistically significant. Therefore, right ventricular hypertrophy in this model appears to be characterized by an essentially well balanced compensatory response of the cytoplasmic components implicated in energy production and energy utilization. In contrast, reduction of the mitochondrial/myofibril volume ratio is a consistent subcellular alteration occurring in left ventricular myocytes following pressure overload hypertrophy.[27]

Studies on the dog heart have shown that occlusion of a major branch of the left coronary artery results in an increased blood flow through the remaining arteries.[30,31] If a similar process occurs in the rat heart, the increased coronary blood flow should reflect a reduction in the resistance of the right coronary artery, due to vasodilation and/or recruitment of more capillaries by the relaxation of precapillary sphincters.[32] The quantitative analysis of the proximal region of the right coronary artery revealed an increase in the mean luminal cross-sectional area (33%) commensurate with the magnitude of ventricular hypertrophy (30%). An even greater growth increment was observed in the vessel wall (54%). The larger luminal area of the coronary artery suggests vasodilatation to accommodate an increased coronary blood flow. On the other hand, such an adaptation is lacking at the capillary level, because the average capillary luminal cross-sectional area and the total capillary luminal volume remained essentially constant. The mechanism by which an increased blood flow through the dilated right coronary artery is achieved without a proportional expansion of the capillary bed is at present unknown. This is a significant issue to be resolved and may require extensive morphometric analysis of the different portions of the coronary vasculature, involving an estimation of collateral channels through which blood flow could be diverted toward the region of the infarct.

In summary, the results of the present study indicate that right ventricular hypertrophy in this model is characterized by a differential growth response of the myocardial components that constitute the tissue, cellular, and subcellular levels of organization. The major findings are as follows: 1) reduction in capillary luminal volume density; 2) reduction in capillary luminal surface density; 3) increase in the maximum diffusion distance for oxygen; 4) dilatation of the right coronary artery commensurate with the magnitude of ventricular hypertrophy; 5) proportional cellular enlargement of the myocyte population; and 6) no significant change in the mitochondria/myofibril volume ratio. It can be concluded, therefore, that the inadequate compensation of the capillary bed results in alterations of the structural properties of the microvasculature implicated in oxygen availability, diffusion, and transport that might be detrimental to the myocardium.

References

1. Norman TD, Coers CR: Cardiac hypertrophy after coronary ligation in rats. Arch Pathol 1960, 69:181–184
2. Turek Z, Grandtner M, Kubat M, Ringnalda BEM, Kreuzer F: Arterial blood gases, muscle fiber diameter and intercapillary distance in cardiac hypertrophy of

rats with an old myocardial infarction. Pflugers Arch 1978, 376:209-215
3. Pfeffer MA, Pfeffer JM, Fishbein MC, Fletcher PJ, Spadaro J, Kloner RA, Braunwald E: Myocardial infarct size and ventricular function in rats. Circ Res 1979, 44:503-512
4. Fishbein MC, Maclean D, Maroko PR: Experimental myocardial infarction in the rat. Qualitative and quantitative changes during pathologic evolution. Am J Pathol 1978, 90:57-70
5. Hort W, da Canalis S, Just H: Untersuchungen bei chronischem experimentellen Herzinfarkt der Ratte. Arch Kreisl-Forsch 1964, 44:288-299
6. Selye H, Bajusz E, Grasso S, Mendell P: Simple techniques for the surgical occlusion of coronary vessels in the rat. Angiology 1960, 11:398-407
7. Lais LT, Rios LL, Boutelle S, DiBona GF, Brody MJ: Arterial pressure development in neonatal and young spontaneously hypertensive rats. Blood Vessels 1977, 14:277-284
8. Anversa P, Olivetti G, Melissari M, Loud AV: Stereological measurement of cellular and subcellular hypertrophy and hyperplasia in the papillary muscle of adult rat. J Mol Cell Cardiol 1980, 12:781-795
9. Mendez J, Keys A: Density and composition of mammalian muscle. Metabolism 1960, 9:184-188
10. Gundersen HJG: Notes on the estimation of the numerical density of arbitrary profiles: The edge effect. J Microsc 1977, 111:219-223
11. Loud AV, Anversa P, Giacomelli F, Wiener J: Absolute morphometric study of myocardial hypertrophy in experimental hypertension: I. Determination of myocyte size. Lab Invest 1978, 38:586-596
12. Anversa P, Olivetti G, Melissari M, Loud AV: Morphometric study of myocardial hypertrophy induced by abdominal aortic stenosis. Lab Invest 1979, 40:341-349
13. Anversa P, Olivetti G, Loud AV: Morphometric study of early postnatal development in the left and right ventricular myocardium of the rat: I. Hypertrophy, hyperplasia and binucleation of myocytes. Circ Res 1980, 46: 495-502
14. Weibel ER: Oxygen demand and the size of respiratory structures in mammals, Evolution of Respiratory Processes. Edited by SC Wood, C Lenfant. New York-Basal, Marcel Dekker, 1979, 13:289-346
15. Hoppeler H, Mathieu O, Weibel ER, Krauer R, Lindstedt SL, Taylor CR: Design of the mammalian respiratory system: VIII. Capillaries in skeletal muscles. Respir Physiol 1981, 44:129-150
16. Olivetti G, Anversa P, Melissari M, Loud AV: Morphometric study of early postnatal development of the thoracic aorta in the rat. Circ Res 1980, 47:417-424
17. Bishop SP, Oparil S, Reynolds RH, Drummond JL: Regional myocyte size in normotensive and spontaneously hypertensive rats. Hypertension 1979, 1:378-383
18. Lin HL, Katele KV, Grimm AF: Functional morphology of the pressure- and the volume-hypertrophied rat heart. Circ Res 1977, 41:830-836
19. Anversa P, Loud AV, Giacomelli F, Wiener J: Absolute morphometric study of myocardial hypertrophy in experimental hypertension: II. Ultrastructure of myocytes and interstitium. Lab Invest 1978, 38:597-609
20. Anversa P, Giacomelli F, Wiener J: Regional variation in capillary permeability of ventricular myocardium. Microvasc Res 1973, 6:273-285
21. Anversa P, Olivetti G, Loud AV: Morphometric studies of left ventricular hypertrophy, Perspectives in Cardiovascular Research. Vol 8. Edited by RC Tarazi, JB Dunbar. New York, Raven Press, 1983, pp 27-39
22. Tomanek RJ: Effects of age and exercise on the extent of the myocardial capillary bed. Anat Rec 1970, 167:55-62
23. Leon AS, Bloor CM: The effect of complete and partial deconditioning on exercise-induced cardiovascular changes in the rat. Adv Cardiol 1976, 18:81-92
24. McElroy CL, Gissen SA, Fishbein MC: Exercise-induced reduction in myocardial infarct size after coronary artery occlusion in the rat. Circulation 1978, 57: 958-962
25. Anversa P, Levicky V, Beghi C, McDonald SL, Kikkawa Y: Morphometry of exercise-induced right ventricular hypertrophy in the rat. Circ Res 1983, 52:57-64
26. Korecky B, Rakusan K: Normal and hypertrophic growth of the rat heart: changes in cell dimensions and number. Am J Physiol 1978, 234:H123-H128
27. Page E, McCallister LP: Quantitative electron microscopic description of heart muscle cells: Application to normal, hypertrophied and thyroxin-stimulated heart. Am J Cardiol 1973, 31:172-181
28. Lehninger AL: Biochemistry: The Molecular Basis of Cell Structure and Function. 2nd edition. New York, Worth, 1975, p 1104
29. Hoppeler H, Mathieu O, Krauer R, Claassen H, Armstrong RB, Weibel ER: Design of the mammalian respiratory system: VI. Distribution of mitochondria and capillaries in various muscle. Respir Physiol 1981, 44: 87-111
30. Herzberg RM, Rubio R, Berne RM: Coronary occlusion and embolization: Effect on blood flow in adjacent arteries. Am J Physiol 1966, 210:169-175
31. Driscol TE, Eckstein RW: Coronary inflow and outflow responses to coronary artery occlusion. Circ Res 1967, 20:485-495
32. Polimeni PI, Al-Sadir J: Expansion of extracellular space in the nonischemic zone of the infarcted heart and concomitant changes in tissue electrolyte contents in the rat. Circ Res 1975, 37:725-732

Acknowledgments

We wish to express our appreciation to Dr. Alden V. Loud for stimulating discussions throughout the study and for the critical review of the manuscript. The expert assistance of Mrs. Maria Demeri, Ms. Laura Matranga, Mr. Alfred E. Revzin, and Mr. Kenneth Katz is gratefully acknowledged.