Capillary Growth in Anemia-Induced Ventricular Wall Remodeling in the Rat Heart

Giorgio Olivetti, Costanza Lagrasta, Federico Quaini, Roberto Ricci, Giuseppe Moccia, Joseph M. Capasso, and Piero Anversa

To determine whether anemia-induced cardiac hypertrophy affects ventricular size and shape and the component structures of the capillary network of the left and right ventricles, young male rats were fed an iron- and copper-deficient diet for 7 weeks. By that time, blood hemoglobin content fell to 5±1 g/dl, and packed cell volume fell to 18±3%. To further characterize the implications of anemia, red blood cell number, hemoglobin corpuscular content, systemic arterial pressure, heart rate, and blood viscosity were measured. Moreover, the changes in ventricular weights were analyzed in terms of the alterations in ventricular wall area and ventricular wall thickness to establish the impact of the elevation in load associated with a high cardiac output state on ventricular remodeling. The quantitative properties of the capillary circulation were also examined biventricularly by low power electron microscopic morphometry to evaluate the adaptive growth potential of the coronary microcirculation in this form of cardiac hypertrophy. Anemia was found to interfere with the production of red blood cells and their mean corpuscular hemoglobin content and resulted in a 40% reduction in blood viscosity and a 12% and 27% decrease in systolic and diastolic blood pressure, respectively. The changes in heart rate were not statistically significant. In comparison with control animals, heart weight increased by 50%, but the enlargement in right ventricular mass (65%) was greater than that of the left ventricle (47%). Ventricular hypertrophy occurred with increases in wall area and wall thickness although the former increased consistently more than the latter in either ventricle. Tissue growth was accompanied by a 60% lengthening of the capillary network, which in combination with an increase in capillary diameter resulted in a 65% and 34% expansion in capillary luminal volume and 56% and 20% larger luminal surface density in the left and right sides of the heart, respectively. In conclusion, hypochromic microcytic anemia leads to eccentric ventricular hypertrophy with a significant amount of capillary proliferation that may tend to protect the myocardium from the increased potential for ischemic injury. (Circulation Research 1989;65:1182-1192)

Hypochromic anemia produces multiple effects on the peripheral circulation and heart function. In chronic and slowly developing forms, cardiac output increases because the decrease in blood viscosity and vascular resistance tends to elevate the preload and lower the afterload. Since myocardial hypertrophy accompanies the evolution of the anemic state, anemia may be well tolerated with no signs of impairment of ventricular performance. The adaptive response of the coronary bed to the anemic condition includes an increase in coronary blood flow and myocardial oxygen extraction and a decrease in minimal coronary vascular resistance. Coronary flow to collaterals is enhanced in combination with the formation of new collateral vessels. The interaction of these functional and structural changes of the coronary circulation with the hemodynamic alterations generating a volume-overload stress on the myocardium may have an important impact on the remodeling of the ventricular wall and its vascular supply. This is particularly relevant because increased blood flow and local hypoxia have been considered to be mediating factors in the process of capillary neogenesis and proliferation. Moreover, it is yet to be demonstrated whether anemia-induced elevated preload provokes a compensatory response of the heart of equal magnitude in both ventricles.
The current study has been undertaken to assess the growth capacity of the left and right ventricular myocardium after chronic severe hypochromic microcytic anemia. The morphological changes in the ventricles have been analyzed quantitatively by measuring the component structures implicated in tissue oxygenation and their mechanism of growth within the ventricular wall. The significance of evaluating these parameters lies in the estimation of the tissue properties that characterize the response of the microcirculation when hypertrophy and a decreased oxygen-carrying capacity of the blood to the tissue are simultaneously present.

Materials and Methods

Study Design

Eighty-one male Wistar rats (Morini Laboratories, Reggio Emilia, Italy) weighing 37±7 g were used in this study. Rats were housed in pairs in cages with a wire mesh floor. During an acclimation period of 1 week, animals received standard pellet chow and water ad libitum and were maintained on a 12-hour dark-light cycle. Each rat was then labeled, weighed, and subjected to the first hematologic profile. Animals were subsequently pooled and divided into four groups: group 1 (n=16) rats were immediately killed to characterize baseline conditions; group 2 (n=13) rats were exposed to a standard pellet chow diet; group 3 (n=23) rats were placed on a diet (see below) supplemented with iron and copper; group 4 (n=29) rats were exposed to the same diet as group 3 animals, but the diet was deficient in iron and copper. Blood analysis of animals of groups 2, 3, and 4 was performed weekly, and the rats were killed after 7 weeks of treatment.

Diet Characteristics

The three diets were obtained from the same source (Piccioni Laboratory, Milano, Italy). The iron- and copper-deficient diet contained 15% protein, 8.5% fat, 73% carbohydrate, less than 10 ppm iron, and less than 2 ppm copper. The supplemented diet was identical in protein, fat, and carbohydrate concentrations, but it included 100 ppm iron and 10 ppm copper. The standard pellet chow diet consisted of 21% protein, 4.8% fat, 61.5% carbohydrate, 240 ppm iron, and 35 ppm copper.

Blood Analysis

One hundred microliters of heparinized blood was collected in conscious rats by puncture of the tail vein. In each sample, hemoglobin blood level, red blood cell counts, mean corpuscular volume, and mean corpuscular hemoglobin content were determined by an automatic analyzer (Coulter Electronics, Hialeah, Florida). Packed cell volume measurements were obtained after blood centrifugation (microhematocrit centrifuge, Drummond Scientific, Broomall, Pennsylvania) for 3 minutes at 14,000g in microhematocrit capillaries (32 mm×0.8 mm). The length of the blood column and the length of the red blood cell column were measured, and packed cell volumes were computed from the ratio of these two determinations.

Blood Viscosity

In nine rats of group 4 and in six rats each of groups 1, 2, and 3, blood viscosity was measured. Approximately 2 ml EDTA-treated blood was collected from the carotid artery just before the rats were killed. Determinations were performed at 37° C with a cone/plate viscometer (Wells-Brookfield, Brookfield Engineering Laboratory, Stoughton, Massachusetts). All measurements were obtained within 10 minutes of blood withdrawal and were expressed in centipoise at a shear rate of 450 sec⁻¹.

Fixation Procedure

Rats were anesthetized intravenously with 5 g/kg body wt fentanyl citrate and 250 g/kg body wt droperidol. The right carotid artery was exposed, cannulated, and attached to a Statham pressure transducer (model P23ID, Gould Instruments, Cleveland, Ohio) connected to a Gould pressure processor amplifier and a Gould ES-1000B electrostatic chart recorder for the measurement of systemic arterial pressure. Heart rate was obtained from the frequency of the incoming pressure waveform. After these determinations, the abdomen was opened, and the abdominal aorta was cannulated with a catheter connected to a perfusion apparatus. In rapid succession, the heart was arrested in diastole by injecting 1 ml potassium chloride through the jugular vein, the chest was opened, the right atrium was cut, and the coronary vasculature was perfused with phosphate buffer at a pressure equal to the in vivo measured mean arterial pressure. After perfusion with buffer, the coronary circulation was perfused with a solution containing 2% paraformaldehyde and 2.5% glutaraldehyde. Subsequently, the heart was removed, and the weights of the right ventricle and left ventricle including the interventricular septum were recorded. Ventricular volumes were determined by dividing their weights by the specific gravity of muscle tissue (1.06 g/ml).

Tissue Sampling

The ventricles were sliced into approximately ten 1-mm thick slices perpendicular to the major axis of the heart from the apex to the base. Wall thickness was estimated by averaging four equally spaced measurements from each of the four middle tissue slices at a magnification of ×25. Ventricular areas were then computed by dividing wall volume by wall thickness. This calculation assumes that the ventricular wall may be treated as a thin sheet. Subsequently, the four middle slices of the free wall of each ventricle were cut radially to obtain 30 tissue blocks extending from the endocardial to the epicardial surface for electron microscopy. The
specimens were postfixed in 1% osmium tetroxide, dehydrated in acetone, and infiltrated and embedded in araldite. The remaining slices of each ventricle were used for dry weight determinations.

Electron Microscopic Morphometry

As discussed in "Results," this analysis was restricted to rats of group 3 (n=9) and group 4 (n=12). Ten plastic-embedded tissue blocks from each ventricle of these two groups of animals were sectioned at a thickness of 1–1.5 μm and were stained with methylene blue and safranin. Four blocks from each ventricle of each animal were trimmed for thin sectioning to obtain four areas of tissue with transversely sectioned myofibers. Low power electron micrographs, eight from each tissue block, were collected and printed at ×6,100 and calibrated with a diffraction-grating replica magnification standard (E.F. Fullam, Schenectady, New York). These micrographs were analyzed morphometrically with a superimposed grid consisting of 140 sampling points and 14 test-line segments each 150 mm long.

The volume fraction of myocardial components (Vv) was measured in 1,344 of these micrographs (group 3=576; group 4=768) by counting the fraction of sampling points (Pp), overlying myocytes, capillary lumen, capillary endothelium, and other interstitial structures:

\[ Vv = \frac{Pp}{140} \]

The number of capillary profiles (Ncap) in the sampled area (A) was also counted to determine their numerical density [N(cap)A]:

\[ N(cap)A = \frac{Ncap}{A} \]

and average cross-sectional area (Acap):

\[ Acap = \frac{V(cap)}{N(cap)A} \]

Length density, length per unit volume of capillaries [L(cap)v], is numerically equal to their measured numerical density, number per unit area, in transverse myocardial sections:

\[ L(cap)v = N(cap)A \]

The surface density of capillary lumen [S(cap)] per unit volume of myocytes [V(m)v] was evaluated by counting the number of intersections (I) between the sampling line (L) and the profiles of luminal endothelial membrane per unit length of sampling line [L(m)] according to the equation:

\[ S(cap)v = \frac{I}{2L(m)} \]

The luminal surface area of capillary endothelium was expressed in relation to the volume of myocytes because these cells constitute the major oxygen-consuming portion of the tissue and also to eliminate the effects of possible variations in the interstitial space. The influence of obliquity in cardiac muscle has recently been discussed with respect to numerical densities of structures, and surface area measurements and specific suggestions for sampling have been made. These criteria have been followed in the present investigation. Correction factors for the effects of compression were applied in the computation of numerical density and size and surface area of capillaries.

The diffusion distance for oxygen (R), represented by the average distance from the capillary wall to the mitochondria of myocytes, was calculated from the capillary profile density and capillary mean cross-sectional area:

\[ R = \frac{1}{\sqrt{\frac{\pi}{\pi} [N(cap)A]}} \]

The transmural number of capillaries (N_tcap) across the ventricular wall (W), that is, the number of capillaries that would be traversed by a thin transmural probe inserted perpendicularly to the surface of the ventricle, was determined from their measured profile numerical density, N(cap)A, and it was assumed that myocardial capillaries were distributed in a hexagonal pattern:

\[ N_tcap = 1.0244 W \cdot \sqrt{\frac{\pi}{\pi} [N(cap)A]} \]

The aggregate length of capillaries in the ventricle [T_L(cap)] was measured from the product of total ventricular myocardial volume (V_T) and the length of capillaries per unit volume, L(cap)v:

\[ T_L(cap) = V_T \cdot L(cap)v \]

In a similar manner, the values of absolute capillary volume and luminal surface in the entire ventricle were evaluated from the products of total ventricular myocardial volume and their respective values per unit volume. The theoretical aspect and practical application of the morphometric procedure summarized above have recently been described in detail.

Data Collection and Statistical Analysis

All morphometric data were collected blindly, and the code was broken at the end of the experiment. Results are expressed as mean±SD. Statistical significance in multiple comparisons was calculated by the Bonferroni method. The unpaired two-tailed Student's t test was used to determine statistical significance of differences between two measurements. Values of p < 0.05 were considered to be significant.

Results

Anemic State

The effects of the three dietary regimens on body weight, hemoglobin blood level, packed cell volume, and mean corpuscular volume are illustrated in Figure 1. The rate of increase in body weight was not affected during the first 5 weeks of exposure to the iron- and copper-deficient diet. However, in the
last 2 weeks average decreases in body weight of 10% and 11% were measured in the anemic rats compared with the two control groups, which were subjected to a supplemented and standard pellet chow diet, respectively.

As a result of the deficiency in iron and copper, blood hemoglobin fell after 7 days and continued to decrease throughout. From a concentration of 12.76±0.68 g/dl, a value of 5.13±1.05 g/dl (*p<0.0001) was reached at 7 weeks (Figure 1). The change in hemoglobin blood level was accompanied by a reduction in packed cell volume from an initial value of 40.9±2.6% to a final value of 18.3±2.9% (*p<0.0001). Similarly, mean corpuscular volume progressively decreased as a function of time. These properties were practically identical in the two control groups, and no demonstrable change occurred with age in either group (Figure 1). Thus, by using an incomplete diet, a severe form of hypochromic and microcytic anemia was obtained with little interference on body growth.

Figure 2 shows red blood cell counts, mean corpuscular hemoglobin contents, and blood viscosity measurements recorded at sacrifice in the four groups of rats. During the 7-week period of the rapid growing phase of the animals, the number of red blood cells per cubic millimeter of blood increased by nearly 50% (*p<0.0001) in rats on a standard (group 2) or supplemented (group 3) diet with respect to the younger untreated controls (group 1). Average hemoglobin content in erythrocytes, however, decreased by 13% (*p<0.0001).

Anemia interfered with both the production of red blood cells and their mean corpuscular hemoglobin concentration, since red blood cell counts in group 4 rats were similar to those found at baseline (group 1). Moreover, mean corpuscular hemoglobin concentration decreased by 46% (*p<0.0001) in anemic rats, from a baseline value of 21.8±1.1 pg to a value of 11.7±2.0 pg at sacrifice. Figure 2 also shows the measurements of blood viscosity. A 40% (*p<0.0001) decrease and a 28% (*p<0.001) decrease in this parameter were found in anemic animals in comparison with age-matched controls and baseline rats, respectively.

**Arterial Blood Pressure and Heart Rate**

Systolic and diastolic pressures decreased in anemic rats. Compared with controls, an average 12% reduction in systolic pressure (*p<0.01) was observed, from a mean value of 125 mm Hg to a value of 110 mm Hg. An even greater decrement was noted in diastolic pressure (−27%, *p<0.001), from a value of 84 mm Hg to a value of 61 mm Hg. These changes resulted in a 21% elevation (*p<0.01) of differential pressure. Mean arterial pressure was also diminished by approximately 18 mm Hg in anemia. Contrary to expectation, heart rate was slightly reduced in anemic rats although the difference from control animals was not statistically significant. This unusual change in heart rate with anemia may reflect the effects of anesthesia during the measurement.
Cardiac Hypertrophy

The weights of the heart, left ventricle including the septum, and right ventricle and their ratios to body weights are shown in Figure 3. The changes in heart weight associated with the normal growing process per se can be evaluated by comparing animals in groups 2 and 3 with those in group 1. It can be seen that in 7 weeks heart weight increased 143% (group 2) and 141% (group 3) and that these gains were the result of a 157% and 151% expansion of the left ventricular mass and a 97% and 112% augmentation of the right ventricular myocardium.

The combined effects of physiological growth and anemia on cardiac weights can be determined by analyzing the differences between group 4 and group 1 rats. Heart weight was 265% greater in anemic animals, which also showed 273% and 237% larger left and right ventricles, respectively. By measuring the changes between anemic rats and their age-matched controls, the magnitude of hypertrophic growth dependent on the influence of hypochromic microcytic anemia on the heart could be assessed. Data showed that heart weight in group 4 animals increased by 51% ($p<0.0001$) compared with group 3 and 50% ($p<0.0001$) compared with group 2.
animals (Figure 3). These changes were due to a 59% \((p<0.0001)\) and 70% \((p<0.0001)\) augmentation of the right ventricle and a 49% \((p<0.0001)\) and 45% \((p<0.0001)\) expansion of the left ventricle. Moreover, none of the small differences between the two control groups were found to be statistically significant; thus, the diet supplemented with iron and copper was comparable with the standard diet in supporting myocardial growth during the 7-week period of administration.

Since body weight of anemic rats was lower than that of controls, the changes in the ratios of heart weight and left and right ventricular weights to body weights were all significantly greater than the increments in absolute cardiac weights (Figure 3). Measurements of the ratio of tissue dry weight to fixed-tissue wet weight were practically identical in control and anemic rats (group 4: left ventricle, 0.257±0.009; right ventricle, 0.237±0.009; group 3: left ventricle, 0.261±0.015; right ventricle, 0.245±0.015); thus, cardiac hypertrophy was not associated with an increase in water content in the muscle of either ventricle.

Figure 4 illustrates how the expansion in myocardial mass affected the shape of the ventricles in this animal model. Maturational growth as a function of age was characterized by a biventricular increase in wall area that exceeded the change in wall thickness. The calculation of ventricular wall area assumes that the ventricular wall may be treated as a thin sheet. Thus, increases in wall area correspond to chamber dilatation. The addition of anemia to physiological development led to a hypertrophic response of the heart that involved an augmentation in wall thickness and wall area in both ventricles. However, the augmentation in ventricular wall area consistently exceeded that in wall thickness; this finding implied a larger chamber volume without a relative increase in wall thickness.

**Capillary Adaptation**

To evaluate the structural characteristics of cardiac hypertrophy after the administration of the
iron- and copper-deficient diet, 12 of the 29 treated animals were randomly chosen, and the left and right ventricular myocardium was analyzed morphometrically. Nine of the 23 rats fed with an identical diet supplemented with iron and copper were used as controls. It should be pointed out, however, that no statistical difference was found between the two control groups in terms of cardiac weights and hematologic characteristics. These data have been illustrated in Figures 1-4.

Data were originally collected separately from the subendocardium and subepicardium of each ventricle to analyze whether a differential response between these two regions of the wall was present after hypochromic microcytic anemia. However, similar results were obtained in the two layers sampled so that the data were combined and considered representative of the entire wall of each ventricle.

The changes in the volume composition of the myocardium as a result of anemia are shown in Figure 5. The volume percent of capillaries in the tissue was seen to increase by 48% in the left ventricle \((p<0.0001)\) and by 30% in the right ventricle \((p<0.0001)\). The volume fraction of capillary lumen, however, increased by 65% in the left ventricle and 34% in the right ventricle. In contrast, the myocyte compartment decreased by 11% \((p>0.0001)\) and 5% \((p<0.001)\) in the left and right sides of the heart, respectively. Moreover, a 31% expansion in the interstitium \((p<0.005)\) of the left myocardium was measured.

Figure 6 illustrates the structural properties of the capillary network implicated in tissue oxygenation. Since these quantitative characteristics are dependent on capillary size and number, the numerical
density of capillary profiles per unit area of myocardium and the capillary luminal mean cross-sectional area are shown first in this figure. Despite a significant amount of cardiac hypertrophy, the concentration of capillaries in the tissue was found not to be affected in either ventricle. However, the transverse luminal area of capillary profiles increased by 54% ($p<0.001$) in the left ventricle and by 32% ($p<0.005$) in the right ventricle. These increases in capillary size were responsible for the measured 56% ($p<0.001$) and 20% ($p<0.05$) augmentation in capillary luminal surface per unit volume of myocytes in the left and right sides of the heart, respectively, and the 14% ($p<0.001$) decrease in the diffusion distance for oxygen in the left ventricular myocardium. The 7% reduction in diffusion distance in the right ventricle was not statistically significant.

As a result of the anemic state, the number of capillary profiles across the ventricular wall increased from 150±35 to 184±41 in the left ventricle and from 55±9 to 68±16 in the right ventricle. These differences, however, were not statistically significantly different.

Figure 7 shows the absolute changes in luminal volume and length and surface of capillaries in each ventricle of control and anemic rats. It can be seen that the iron- and copper-deficient diet resulted in a 120% and 106% increase in volume, a 59% and 60% lengthening of the entire capillary network, and a 105% and 80% expansion in surface in the left and right myocardium, respectively.

Discussion

Anemia

The results of the present investigation indicate that young adult rats fed an iron- and copper-deficient diet developed over a period of 7 weeks a severe hypochromic microcytic anemia, which became apparent after 7 days of treatment and worsened with time. The composition of the diet minimized the effects of malnutrition on body growth.17 Although previous studies of nutritional anemia in young and adult rats have used comparable deficiencies of iron and copper intake with similar characteristics of the anemic state,18-22 the rate of body growth was consistently impaired, and the influence of malnutrition could not be fully excluded. Moreover, direct comparisons of changes in heart weight and its major subdivisions could not be made because of the large differences in body weight between treated and untreated animals.18,20

In this report the decrease in size of red blood cells and packed cell volume produced a significant reduction in blood viscosity and consequently an increase in volume load on the heart due to the diminished peripheral vascular resistance.1,2 The alteration in the corpuscular component of circulating blood resulted also in a decrease in systemic arterial pressure and an elevation in differential pressure demonstrating a greater impact of anemia on the diastolic properties of the cardiovascular system. Moreover, the hemoglobin content in erythrocytes as well as the production of red blood cells diminished; these decreases led to a reduction in the oxygen-carrying capacity of the blood.

Cardiac Hypertrophy

When an increase in work load on the heart is imposed during a phase of active growth of the
animals, the myocardial adaptation involves responses that reflect the combined effect of physiological growth with age and the overload per se. Since arterial blood pressure remains essentially constant in young adult rats, the expansion of cardiac mass in animals exposed to a diet adequate in iron and copper had to be the result of an increase in circulating blood volume due to the gain in body weight. Although the changes in weight of the left ventricle were slightly greater than those of the right ventricle, both sides of the heart were characterized by an augmentation in wall area with a moderate thickening of the wall. These findings were consistent with an elevated volume-load stress on the myocardium and eccentric hypertrophy. Similarly, anemia superimposed on maturational growth produced an analogous type of response that was, however, greater in magnitude. Thus, anemia-induced cardiac hypertrophy can be seen as a form of accelerated myocardial growth in which the rate of increase in wall area exceeds that in ventricular wall thickness. This conclusion is supported by the larger wall area measured biventricularly in anemic rats with respect to their age-matched controls.

The findings of the present report also indicate that after hypochromic microcytic anemia the heart responds by different magnitudes of tissue growth of the right and left ventricular myocardium. The change in mass of the right ventricle was consistently larger than that of the left ventricle; the right ventricle averaged a 13% greater accumulation of tissue. The differential growth rate between the two ventricles is in agreement with the higher cardiac output of anemic rats observed as early as 24 hours after the induction of anemia. Although the primary stimulus is volume overload, however, the elevation in diastolic wall stress should be relatively greater per unit volume of tissue in the right ventricle because of its smaller total number of myocytes, less ventricular mass, and thinner wall. A similar mechanism for the greater capacity of left ventricular myocardium to accommodate a heightened work load has been previously noted in chronic dynamic exercise in rodents although these observations are not in complete agreement with other reports of endurance training in rats. Moreover, the biventricular response found in anemia is similar to that observed in other animal models of high cardiac output states such as those associated with thyroxine administration and an arterial venal shunt.

Ventricular growth in volume hypertrophy occurs with enlargement of the ventricular chamber as shown here by the increase in wall area that exceeded the change in wall thickness. Chamber enlargement has been shown to be brought about through lengthening of myocytes; this lengthening is most likely caused by replication of sarcomeres in series. Cell lengthening would counteract the greater end-diastolic wall stress by contributing to the enlargement in chamber volume that would otherwise have to occur by spatial rearrangement or lateral slippage of myocardial fibers within the wall, or both. Such a possibility may be implicated in the dilation of the ventricular chamber in the failing heart. Capillary Growth

The morphometric analysis of the myocardium indicates that anemia-induced hypertrophy is accompanied by a significant amount of capillary proliferation in the left and right ventricles. The process of capillary neogenesis resulted in an average 23% increase in the number of capillaries across the wall biventricularly, but this change was not statistically significant. Although an increase of this parameter with myocardial enlargement would unequivocally demonstrate capillary proliferation, augmentations in wall capillaries can be considered to be only minimal indexes of capillary proliferation because they do not take into account the overall expansion in ventricular mass. Thus, the estimation of total capillary length in the ventricle offers a direct measurement of absolute capillary growth. In anemia, an approximately 60% lengthening of the entire capillary network was measured in both ventricles. On the other hand, this change in the aggregate length of capillaries may reflect an overestimation of capillary proliferation since the mean length of a capillary unit in the myocardium of a normal or anemic rat is at present unknown. The magnitude of capillary neogenesis observed here after anemia, however, may reflect, in part, the young age of the animal at which the anemic state was imposed. Maturation and aging have been shown to be important components in the vascular and myocyte responses of the heart to stressful conditions.

Studies dealing with the adaptation of myocytes in anemia have shown that the mitochondrial fraction in the cell cytoplasm increases through the addition of new mitochondria of the same size. The myofibrillar compartment, however, remains unchanged maintaining its capacity to grow when an added work load is imposed on the ventricle. Moreover, the correction of anemia rapidly restores the composition of myocyte cytoplasm to control values. Mechanisms controlling vascular growth in the mammalian heart are poorly understood. In skeletal muscle, hypoxia, high blood flow, and chronic vasodilatation all appear to be capable of stimulating angiogenesis in normal and pathological states. In anemia these three factors are simultaneously present. Since the reduced oxygen-carrying capacity of the blood may generate local hypoxia, coronary blood flow is elevated, and vasodilatation may exist in view of the reduced coronary vascular resistance and the increase in luminal cross-sectional area of the capillary network measured here. In this regard, chronic
hypoxia after exposure to simulated high altitude or reduced-oxygen tension has been found to induce cardiac hypertrophy and capillary proliferation. However, it cannot be excluded that the release of humoral substances in the interstitium may play an additional important role in the process of capillary proliferation.

Capillary neogenesis in cardiac hypertrophy may be necessary to maintain an adequate blood supply to the rapidly expanding ventricular mass and to protect the myocardium from the vulnerability to ischemic injury. In the present study, the volume fraction of capillaries in the tissue and the capillary luminal surface per unit volume of myocytes increased in anemic rats. These structural properties of the capillary bed are functionally related, respectively, to the volume of capillary blood available for gas exchange within the tissue and the capillary area available for oxygen transport from the blood to the tissue. Moreover, the distance from the capillary wall to the surrounding myocytes, which corresponds to the diffusion path length to the sites of oxygen consumption and ATP synthesis, was found to be decreased in the left ventricle. Each of these changes would tend to ameliorate the capacity for tissue oxygenation of the hypertrophied myocardium in anemia. In contrast, these compensatory mechanisms have not been consistently observed in several models of pressure hypertrophy and volume hypertrophy or after a combination of pressure- and volume-overload stress (for review, see Reference 24).

In conclusion, hypochromic microcytic anemia leads to eccentric ventricular hypertrophy, which is characterized by a significant amount of capillary proliferation. Capillary growth may constitute an adaptive compensatory response of the tissue to counterbalance the reduced oxygen-carrying capacity of the blood and the increased potential for ischemic injury.

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