Effect of anemia on cardiac function, microvascular structure, and capillary hematocrit in rat hearts

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Received 8 August 2000; accepted in final form 31 October 2000

Rakusan, Karel, N. Cicutti, and F. Kolar. Effect of anemia on cardiac function, microvascular structure, and capillary hematocrit in rat hearts. Am J Physiol Heart Circ Physiol 280: H1407–H1414, 2001.—The effect of anemia on the coronary microcirculation was studied in young male rats. Chronic anemia resulted in increased left ventricular end-diastolic pressure and decreased functional reserve. Cardiac mass in anemic animals increased by 25%. Capillary and arteriolar densities in these hearts remained unchanged, indicating angiogenesis in this experimental situation (estimated aggregate capillary length in the left ventricle of anemic hearts was 3.06 km compared with 2.35 km in control hearts). Capillary hematocrit was decreased in chronic anemia less than systemic hematocrit: from 25 to 18% in anemia versus 45 to 28% in controls. Capillary hematocrit and red blood cell spacing were also studied after acute blood withdrawal. Here, capillary hematocrit was preserved even more: 22 versus 24% in systemic hematocrit. Finally, the same was studied in isolated hearts perfused with solutions of various hematocrits. After perfusion with low-hematocrit solution (14%), the capillary hematocrit (24%) was even higher than the perfusate hematocrit! In conclusion, we found evidence of angiogenesis in cardiomegaly induced by chronic anemia. Microvascular growth was accompanied by advantageous regulation of red blood cell spacing within these vessels. This was even more pronounced during acute hemodilution and in isolated perfused hearts.

CHRONIC ANEMIA is usually accompanied by an increase in cardiac mass due to volume overload. Cardiomegaly, in turn, may be associated with a deficit in vascular supply unless the increase in the volume of cardiac myocytes is associated with an adjusted vascular growth. Both the decrease in arterial blood oxygen content as well as vascular deficit may lead to impaired oxygen delivery to myocardial tissue. One of the principal aims in this series of experiments was to study the effect of experimental iron-deficient anemia on cardiac function and coronary microvascular structure. We hypothesized that chronic anemia would lead to coronary angiogenesis and microcirculatory restructuring as a response to hypoxia and/or increased coronary blood flow due to reduced blood viscosity, both being recognized as powerful angiogenic stimuli (11, 24).

Under certain conditions, the capillary blood ceases to be a continuous source of oxygen. This is because the red blood cells (RBCs) travel within capillaries mostly in single file separated by plasma gaps of variable lengths. Theoretical studies (5, 8, 9) have demonstrated that as the distance between RBCs increases, a constant O₂ flux at the capillary midway between two blood cells cannot be maintained and O₂ conductance is reduced. This would be even more pronounced in situations associated with decreased hematocrit when the RBC separation distances increase. Therefore, we decided to study coronary capillary hematocrit in rats with chronic anemia. We hypothesized that decreases in the capillary hematocrit would be less pronounced than the large vessel hematocrit to minimize the deleterious effect of increased RBC spacing.

Capillary hematocrit in rat hearts with chronic anemia can be influenced by various adaptational mechanisms associated with remodelling of cardiac tissue, which may also include structural alterations of the microvascular bed (thickness of arteriolar wall, capillary segment diameter and length, branching angle, preferential channels, etc.). Alternatively, neurohumoral modulation at the level of terminal arteries, arterioles, and capillary sphincters may, at least in part, be responsible for the associated changes. In an effort to distinguish between these possible effects, we compared capillary hematocrit values in hearts from rats with chronic anemia with those obtained from rats with an acute decrease in hematocrit and also from isolated rat hearts perfused with blood of various hematocrit values.

Thus, in the present study, we investigated the effect of chronic anemia on cardiac function in the rat heart, which was followed by the morphometric analysis of the coronary microvascular structure. Finally, RBC spacing in the coronary capillaries and capillary hematocrit was determined in hearts from rats with...
chronic anemia, and the results were compared with those obtained from hearts of rats after acute hemodilution in vivo or in vitro.

METHODS

Experimental design. The experimental design consisted of three experimental models on Sprague-Dawley male rats: 1) chronic sideropenic anemia, 2) acute hemodilution in vivo, and 3) acute hemodilution in vitro.

Chronic sideropenic anemia. Chronic sideropenic anemia was introduced in Sprague-Dawley male rats weaned on day 26. Animals were kept for an additional 4–5 wk and fed either a standard laboratory diet in control animals or an iron- and copper-deficient diet (TD 80396, Harlan Teklad; Madison, WI) in the anemic group. All animals were weighed daily, and their hemoglobin concentration and hematocrit values were measured from the samples of tail blood at the beginning, in the middle, and at the end of experiment. Both anemic rats and their control littermates were equally divided for functional studies, for morphometric analysis of the cardiac microvasculature (in which fixed myocardial tissue was used), and for determination of RBC spacing and additional morphometric measurements based on frozen material.

For the studies using fixed tissue, the rats were anesthetized with pentobarbital sodium (52 mg/kg ip), and their hearts were perfused in situ with heparinized saline and fixed with 1.5% glutaraldehyde buffered to pH 7.4 with phosphate buffer. Subsequently, the hearts were removed and trimmed of fat and fibrous tissue, and the ventricles were separated from the atria and weighed. The left ventricle and septum were cut at one-third of the distance from the base to the apex of the heart, and samples from this region were subsequently used for detailed morphometry.

Frozen samples were taken from anesthetized (pentobarbital sodium) rats as described previously (31). Briefly, potassium-arrested hearts were frozen in situ by encasing the heart with precooled copper freezing clamps designed to match the shape of the heart, excised while still in contact with the clamps, and submerged in liquid N2. Hearts were stored at −86°C until cryostat sectioning. Small portions of the apical region were used for tissue dry weight determination. In this case, samples were initially weighed (wet weight), dried in an oven (60°C) for more than 36 h, and reweighed (dry weight), and the ratio of wet weight to dry weight was recorded.

Acute hemodilution in vivo. Acute hemodilution in vivo was produced in adult male rats (320–370 g body wt). They were anesthetized as in Chronic sideropenic anemia, and the left femoral vein and both of the carotid arteries were cannulated. Heparin (Sigma) was injected via the femoral vein at a dose of 200 IU/300 g body wt. Blood was withdrawn from the right carotid artery and, simultaneously, the same volume of 5% BSA (Sigma) dissolved in saline was infused into the femoral vein. The rate of withdrawal and infusion was 1 ml/min. To obtain similar mean and variation of hematocrit values as in the chronic anemia group, the total volume of blood exchange ranged from 2.6 to 3.9 ml/100 g body wt. During the experiment, blood pressure in the carotid artery was measured by a conventional pressure transducer (Gould P23 ID) connected to a Grass Polygraph and PC computer. Heart rate was calculated from the pressure signal. At 15 min after isovolemic hemodilution, a blood sample was taken from the tail for the measurement of hematocrit and hemoglobin concentrations, and the heart was stopped with saturated KCl via the femoral vein and frozen as described above.

Control animals were subject to the same procedures except for hemodilution.

Acute hemodilution in vitro. Adult male rats (240–270 g body wt) were killed by pentobarbital sodium overdose, and their hearts were rapidly removed and perfused according to Langendorff under nonrecirculating conditions by a Krebs-Henseleit solution containing the following (mmol/l): 118.0 NaCl, 4.7 KCl, 1.25 CaCl2, 1.2 MgSO4, 25.0 NaHCO3, 1.2 KH2PO4, 7.0 glucose, and 2.0 sodium pyruvate. The medium was gassed with 95% O2–5% CO2 (pH 7.4) and maintained at 37°C. Coronary flow was kept constant at −7.5 ml/min.

Donor animals (420–470 g body wt) were anesthetized and heparinized as in Chronic sideropenic anemia. The left femoral vein and left carotid artery were cannulated. After 3 min of the initial perfusion with the Krebs-Henseleit solution (blood washout), the side branch of the aortic cannula was connected to the cannula in the carotid artery of the donor animal and, simultaneously, the inflow of the Krebs-Henseleit solution was stopped or reduced. The heart was perfused for an additional 4 min. Subsequently, the effluent blood sample was taken for the measurement of hematocrit and hemoglobin, and the heart was freeze-clamped and stored at −86°C.

The loss of blood in the donor rat was compensated by an infusion of blood into the femoral vein, which was previously withdrawn from another anesthetized animal. In some rats, either the blood variably diluted with 5% BSA in saline or 5% BSA in saline alone was infused to the donor. By a combination of these manipulations, we were able to achieve hematocrit values in the perfusate ranging from 7 to 44%. On the basis of hematocrit values in the perfusate, the hearts were divided into three experimental groups: near-normal blood hemocrit (34–44%), moderately reduced hematocrit (23–33%), and low hematocrit (7–21%).

During both initial perfusion with the Krebs-Henseleit solution and subsequent perfusion with full or diluted blood, the coronary flow and perfusion pressure were recorded. Coronary flow was measured by a timed collection of coronary effluent, and the mean perfusion pressure in the side branch of the aortic cannula was measured by a pressure transducer (Gould P23 ID) as above.

Evaluation of left ventricular function. Blood pressure in the left ventricle of anesthetized (pentobarbital sodium, 50 mg/kg ip) rats was measured using a Millar catheter-tip transducer connected to a Grass Polygraph and a PC computer. The catheter was inserted into the left ventricular cavity via the right carotid artery under continuous pressure monitoring. Baseline measurements were recorded after a stabilization period of 10 min; the mean from three consecutive recordings was calculated.

The analog pressure signal was digitized with a sampling frequency of 1 kHz and stored on computer for later processing. The following parameters were derived using our computer program: left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), left ventricular developed pressure (LVPD), and the maximal rates of pressure development (+dP/dtmax) and fall (−dP/dtmax). In addition, the time constant of relaxation was calculated on the basis of an exponential model of isovolumetric pressure decay as the time required for the pressure at −dP/dtmax to be reduced by 1/e (18). Heart rate was calculated from the pressure signal.

After baseline parameters were recorded, the maximal isovolumetric performance of the left ventricle was assessed in open-chest animals on the basis of a previously described method (4). A tracheostomy was performed, and the animals were connected to a Harvard rodent ventilation pump (70
strokes/min). The sternum was opened by a lateral incision above the base of the heart, and a ligature was placed on the ascending aorta. Acute clamping of the aorta served as a loading test for ventricular function. The pressure signal was recorded during the 10 s after the clamp and stored on computer. The LVSP, LVDP, and \( \frac{+dP}{dt}_{\text{max}} \) were derived, and their means were calculated from regular beats per each second after the clamp. This approach significantly reduced the influence of rhythm disturbances induced by the clamp. The highest mean values of these parameters were considered to represent maximal isovolumetric performance of the ventricle. The difference between the values after and before the aortic clamp was considered as the functional reserve.

Morphometric analysis of fixed myocardial tissue. The histological methods have been described previously (7, 26). Briefly, after fixation and dehydration in alcohol, the samples were embedded in historesin. The middle portions of the free ventricular walls were used for morphometry. Sections (1 \( \mu \)m thick) were stained by Avallone’s modification of the Jones silver methenamine method for staining basement membranes. This staining procedure gives a sharp black edge to basement membranes and, in this way, facilitates identification of capillaries, muscle fibers, and arterioles within tissue cross sections.

With the use of an Image Analyzer (Bioquant Meg V), the numbers of capillaries, arterioles, and myocytes per unit area of a cross section were determined, enabling us to calculate their respective numerical densities. Subsequently, the myocyte-to-capillary ratio was computed.

Coronary arterioles were analyzed using an Image Analyzer as follows. First, the total area of the cross section was measured. Afterward, all small arteries and arterioles were registered irrespective of the sectioning angle. For each arteriolar profile observed, the shortest (minimum) and longest (maximum) external diameter were measured together with the minimum internal diameter. These measurements enabled us to estimate the distribution of arterioles according to their size, to measure the arteriolar thickness, and to estimate the arteriolar length density (average length of arterioles per volume of tissue). Arteriolar length density was calculated on the basis of arteriolar numerical density and the ratios of the long to short axes from each arteriolar profile (1, 3).

Finally, the same tissue was also used for estimation of mast cell density. Mast cells were counted in 3-\( \mu \)m-thick sections stained by modified Nacht’s azure eosin method. Four to six cross sections of the left ventricle and septum were evaluated per heart using an Image Analyzer. (For details, see Ref. 28.)

Studies on frozen myocardial tissue. Samples of frozen myocardial tissue were used for additional morphometric studies and studies of RBC spacing within the capillaries and determination of capillary hematocrit.

Frozen sections provided us with an opportunity to use differential staining, which allows us to distinguish between the proximal (arteriolar) and distal (venular) portions of the capillary bed; the staining protocol has been described elsewhere (26). Briefly, tissue sections (16 \( \mu \)m) were incubated in a solution sensitive to dipeptidyl peptidase IV, which stained the distal portions of the capillaries red. Next, the sections were transferred to a solution sensitive to alkaline phosphatase, which stained the proximal portions of capillaries blue. This approach was used for determination of the capillary domain, an index of the heterogeneity of capillary spacing and capillary segment length.

A capillary domain is defined as the tissue cross-sectional area that is closer to a given capillary than to any other. It was derived in the following fashion. First, the positions of the center of all capillaries in the field were recorded by an Image Analyzer as pairs of coordinates. Perpendicular lines were then drawn in the middle between the capillary whose domain was being calculated and all neighboring capillaries. The lines intersect and form a polygon, the actual capillary domain. This is illustrated in Fig. 1. Measurements were done in each heart on ~600 capillaries in cross sections from subendocardial portions of the left ventricular free wall. Distribution of capillary domain areas was log normal. Therefore, the standard deviation of this log-normal distribution, SD log, was used as an index of the heterogeneity of capillary spacing. The higher the index, the more variable is the capillary spacing and vice versa. Longitudinally cut midsections were used for measuring capillary segment length, defined as the distance between two consecutive branching points (400–600 per heart). Finally, the capillary supply unit was calculated, which is the smallest tissue supply volume that can be modeled in three dimensions (25). It is defined as the product of the average capillary domain area and average capillary segment length. The use of our double-staining method enabled us to distinguish between the capillary supply units derived from the proximal and distal portions of the capillaries.

Finally, 16-\( \mu \)m sections were used for the determination of RBC spacing and capillary hematocrit, as described in detail previously (31). In this case, sections were incubated for 40 min in Papanicolau OG-6, which stains RBCs orange, and the double staining of capillaries (as described above) followed. In these experiments, based on triple staining, the transition from blue to red in capillary walls was often poorly defined, and, therefore, we decided not to distinguish between these two portions of the capillary bed. Accordingly, all the measurements are related to the total capillary length. With the use of the Image Analyzer (Bioquant Meg V), the measurements were done manually in a blind fashion using longitudinally cut midsections of the anterior left ventricular wall for the linear capillary hematocrit, defined as the aggregate RBC length per total capillary length. Total capillary length, of course, included also capillaries perfused by plasma only that were clearly stained by our method. These values were then compared with large vessel hematocrit. In capillary

Fig. 1. An example of computer-derived capillary domains on a tissue cross section.
segments with two or more RBCs, the edge-to-edge distance between two adjacent RBCs was measured. Mean RBC spacing values were determined from 400–500 individual measurements. A RBC spacing value was not measured if capillary branching was present between two RBCs or if a RBC was located within a branching point. Two extreme RBC spacing patterns were also analyzed: the frequency of occurrence of RBC spacing values equal to 0 μm (representing “touching” RBCs) and RBCs spacing values exceeding 40 μm (representing extremely large values) were both recorded.

Statistics. Results are expressed as means ± SE. They were evaluated by a simple unpaired t-test or by one-way ANOVA with subsequent Bonferroni post hoc tests when applicable (more than two comparisons).

RESULTS

The development of body mass and hematocrit in three groups of chronic anemia and their respective controls are depicted in Fig. 2. The low-iron diet in this experimental design did not significantly influence total body mass growth but resulted in an ~50% decrease in hematocrit compared with the age-matched group (P < 0.0001), whereas a smaller but significant increase in hematocrit with age was found in growing control animals. The decrease in hemoglobin concentration in anemic rats was of the same degree as the decrease in hematocrit. The animals used for the three different sets of experiments as described in Experimental designs had almost identical mean values, indicating high reproducibility of the data.

Functional data are summarized in Table 1. Chronic anemia resulted in a significant increase of the LVEDP (P < 0.05). The remaining baseline hemodynamic parameters did not differ from those in control animals. In contrast, all the indexes of maximal mechanical performance of the left ventricle as well as of its functional reserve were significantly reduced in the anemic group (P < 0.01 and P < 0.05, respectively); see Fig. 3.

Cardiac mass (right ventricle and left ventricle free wall + septum) increased by 25% (P < 0.0001); the increase being approximately the same in both ventricles. Relative cardiac mass increased by 29% (see Table 2). The percentage of myocardial tissue dry weight was higher in control than anemic hearts (25.7 ± 0.2 and 24.7 ± 0.2%, respectively, P < 0.001). Table 2 also contains data on capillary and myocyte densities in both groups, which were approximately the same in the case of capillary density, whereas myocyte density was slightly (not significantly) decreased in the anemic hearts. Thus the capillary-to-myocyte ratio increased significantly from 1.18 to 1.33 (P = 0.003).

The capillary bed was evaluated also on frozen sections, which allowed us to distinguish between the proximal (arteriolar) and distal (venular) portions of capillary bed. We confirmed previously reported differences between the proximal and distal portions: proximal capillaries supplied a larger tissue area on a cross section (capillary domain), and capillary segment length and capillary supply unit (domain times segment length) were significantly larger in proximal capillaries (P < 0.001). On the other hand, we found no differences between sections from control and anemic hearts (see Table 3). Similarly, we did not detect any morphometric differences between these two groups in quantitative analysis of the arteriolar bed (Table 4).

Finally, our results on RBCs within the microvascular bed are summarized in Table 5 and Fig. 4. In our in

Table 1. Baseline left ventricular function

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Anemia</th>
<th>P Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>12</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Systolic pressure, mmHg</td>
<td>139 ± 5</td>
<td>134 ± 4</td>
<td>NS</td>
</tr>
<tr>
<td>End-diastolic pressure, mmHg</td>
<td>5.0 ± 4</td>
<td>6.9 ± 0.6</td>
<td>0.05</td>
</tr>
<tr>
<td>Developed pressure, mmHg</td>
<td>135 ± 5</td>
<td>128 ± 4</td>
<td>NS</td>
</tr>
<tr>
<td>+dP/dt_max, mmHg/s</td>
<td>8,007 ± 274</td>
<td>7,441 ± 218</td>
<td>NS</td>
</tr>
<tr>
<td>−dP/dt_max, mmHg/s</td>
<td>6,477 ± 307</td>
<td>6,038 ± 213</td>
<td>NS</td>
</tr>
<tr>
<td>Constant γ, ms</td>
<td>9.5 ± 0.5</td>
<td>8.9 ± 0.5</td>
<td>NS</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>387 ± 9</td>
<td>389 ± 10</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of rats in each group. +dP/dt_max and −dP/dt_max maximal rates of pressure development and fall, respectively; γ, time constant of relaxation; NS, not significant.

Fig. 2. Changes in body mass (A) and systemic hematocrit (Hct) (B) during the experiment in three control (C1, C2, and C3) and three experimental (anemic) (A1, A2, and A3) groups.
vivo experiments using acute blood withdrawal, we succeeded in obtaining the same systemic hematocrit values as in rats with chronic anemia (24.3 and 24.9%, respectively). Both were significantly lower than control values (45%). Capillary linear hematocrit was significantly lower than the systemic hematocrit in all three groups; the difference was less pronounced in the anemic groups: thus capillary-to-systemic hematocrit increased from 0.63 in control to 0.74 in chronic anemia and 0.90 in acute anemia. The differences among the groups were significant at $P < 0.01$. This was accompanied by commensurate results in the RBC spacing and in the percentage of RBC spacing with extreme values (RBC spacing equaling 0 μm or exceeding 40 μm).

Isolated perfused hearts were characterized by relatively high linear capillary hematocrit. Hearts perfused with near-to-normal and moderately low hematocrit solutions had capillary hematocrit values similar to those in the perfusate, and in hearts perfused with very low perfusate hematocrit, the capillary hematocrit was strikingly higher (88% increase). Once again, all the remaining parameters as enumerated above confirm these findings.

**DISCUSSION**

Our results clearly show that young rats fed with a low iron and copper diet develop severe hypochromic anemia within 4–5 wk. The diet was otherwise well balanced, with little influence on body growth. These results are reproducible as documented by identical outcome in three series of experiments. Our results are in agreement with several previous reports (19, 20, 22) on the effect of a low iron and copper diet on blood parameters as well as on body growth responses. For instance, Olivetti and co-workers (19) reported in young rats under identical conditions a similar decline in the hematocrit and hemoglobin values and a small retardation in body growth, which became statistically significant only after 6 wk of treatment. The increase in cardiac mass in our anemic animals was 25%, which was evenly distributed in the right and left ventricles. This significant increase in cardiac mass was smaller than the increase reported on previous studies mentioned above. These animals, however, were kept longer on the iron-deficient diet.

Measurements of baseline functional data in the left ventricle did not reveal any significant differences except for a small but significant increase in LVEDP, which probably reflects increased filling of the ventricle in anemic animals. Textbook characterizations of anemia also describe an increase in heart rate in this situation, but we did not find tachycardia in our anemic animals.

### Table 2. Cardiac mass, capillary, myocyte, and mast cell densities

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Chronic Anemia</th>
<th>$P$ Values</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cardiac mass, mg</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Both ventricles</td>
<td>$801 \pm 14$</td>
<td>$1,001 \pm 22$</td>
<td>0.0001</td>
</tr>
<tr>
<td>Right ventricle</td>
<td>$178 \pm 5$</td>
<td>$220 \pm 6$</td>
<td>0.0001</td>
</tr>
<tr>
<td>Left ventricle and septum</td>
<td>$623 \pm 12$</td>
<td>$782 \pm 11$</td>
<td>0.0001</td>
</tr>
<tr>
<td><strong>Cardiac-to-body mass ratio, g/100 g</strong></td>
<td>$0.279 \pm 0.002$</td>
<td>$0.360 \pm 0.004$</td>
<td>0.0001</td>
</tr>
<tr>
<td><strong>Densities, N/mm²</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Capillaries</td>
<td>$3,443 \pm 71$</td>
<td>$3,565 \pm 84$</td>
<td>NS</td>
</tr>
<tr>
<td>Myocytes</td>
<td>$2,928 \pm 102$</td>
<td>$2,687 \pm 97$</td>
<td>NS</td>
</tr>
<tr>
<td>Mast cells</td>
<td>$3.37 \pm 0.38$</td>
<td>$4.05 \pm 0.24$</td>
<td>NS</td>
</tr>
<tr>
<td>Capillaries/myocytes</td>
<td>$1.18 \pm 0.02$</td>
<td>$1.33 \pm 0.04$</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Values are means ± SE, 9 observations per group.

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Fig. 3. Left ventricular maximal systolic and developed pressure and the maximal rate of pressure development (+dP/dt$_{max}$) and their respective reserves in control (normal) and experimental (anemic) animals. Values are means ± SE.
mic animals. It suggests that an increased cardiac output is maintained mainly by elevated stroke volume in this experimental model. This observation is not surprising because several previous authors (e.g., Refs. 21 and 30) failed to find an increase in the heart rate associated with various models of experimental anemia. Presence of cardiac hypertrophy and ventricular remodeling in anemic animals is a probable cause for the decline in maximal values of LVSP, LVDP, and dP/dt achieved after loading as well as the decrease in their respective reserves. A direct effect of iron and/or copper deficiency in this experimental situation cannot be excluded.

Capillary density (see Table 2) was essentially the same in both anemic and control groups. This is in agreement with previous studies (19, 20, 22) on cardiomegaly induced by experimental anemia in young growing animals. Only Martin and co-workers (15) reported a decreased intercapillary distance in the hearts from near-term ovine fetuses with anemia induced by daily isovolemic hemorrhage, which was accompanied by an induction of hypoxia-inducible factor 1 and vascular endothelial growth factor.

Indexes of capillary supply in our experiments remained similar in both groups even after distinction between the proximal and distal capillaries: capillary domain area, segment length, and capillary supply unit were the same in both experimental groups, whereas the well-known differences between the proximal and distal portions of the capillary bed were preserved (25, 26). Thus domain area, capillary segment length, and capillary supply units were significantly increased in the proximal portions of the capillary bed. No differences in arteriolar parameters were found in comparisons of these two experimental groups.

This is, to our knowledge, the first detailed morphometric analysis of arterioles in anemic cardiomegaly.

A similar capillary and arteriolar density despite a significant increase in cardiac mass in anemic animals is an indirect sign of angiogenesis in this experimental situation. This is also supported by our findings of a significant increase in the capillary-to-myocyte ratio and by a trend toward high values in the percentage of endothelial nuclei labeled with proliferating cell nuclear antigen in anemic hearts. Potential angiogenesis may also be revealed by estimation of the total capillary length in the left ventricles. Assuming 1.06 for the tissue specific gravity and 1.1 as the correction factor for the degree of anisotropy (28), we can calculate the aggregate capillary length in the left ventricle in the control animals as 2.35 km, which increased to 3.06 km in anemic hypertrophy, representing an additional 30% of the total aggregate capillary length. Potential angiogenic stimuli in this particular experimental situation could be anemic hypoxia and/or mechanical factors resulting from capillary dilation and increased capillary blood flow as well as a possible combination of these two angiogenic stimuli (11, 24).

Our findings of lower capillary linear hematocrit in the coronary vascular bed compared with the systemic capillaries is in agreement with many previous studies (for a review, see Ref. 31). RBC spacing values found in the left ventricles of control rats are almost identical to our results reported previously (31) and are larger than the values for the rat atrial capillaries reported by Yamakawa et al. (33). Several previous studies reported that, after hemodilution, the microvascular hematocrit in various tissues is not reduced as much as the systemic hematocrit (e.g., Refs. 12–14, 16, and 17), whereas one study reported a larger decrease in capillary hematocrit than in systemic (23), and one study found the same changes in both portions of the vascular bed (27). Our study, which compares situations in the coronary microvascular bed in acute and chronic anemia in vivo as well as in isolated hearts perfused with solutions of various hematocrit values, has, to our knowledge, original observations. Significant differences in the capillary hematocrit and RBC spacing values were found among these various experimental conditions. The main feature is a tendency to maintain a normal or close-to-normal capillary hematocrit vis-à-vis the decreased hematocrit in the large vessels or in the perfusate itself. The most remarkable results are in

### Table 3. Frozen tissue: capillary morphometry

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Anemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Domain area, μm²</td>
<td>378 ± 10*</td>
<td>396 ± 23*</td>
</tr>
<tr>
<td>Log SD × 1,000</td>
<td>61 ± 2</td>
<td>61 ± 3</td>
</tr>
<tr>
<td>Capillary segment length, μm</td>
<td>94 ± 4†</td>
<td>96 ± 7†</td>
</tr>
<tr>
<td>Capillary supply unit, μm³</td>
<td>35,591 ± 1,922†</td>
<td>38,308 ± 4,381†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 16 control rats and 18 anemic rats. Significant differences from venular: *P < 0.01 and †P < 0.001.

### Table 4. Fixed tissue: Arterioles

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Anemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Numerical density, n/mm²</td>
<td>4.6 ± 0.4</td>
<td>4.4 ± 0.2</td>
</tr>
<tr>
<td>Length density, mm/mm²</td>
<td>13.5 ± 1.4</td>
<td>13.0 ± 0.6</td>
</tr>
<tr>
<td>External diameter, μm</td>
<td>15.6 ± 0.5</td>
<td>15.8 ± 0.4</td>
</tr>
<tr>
<td>Internal diameter, μm</td>
<td>7.3 ± 0.3</td>
<td>7.2 ± 0.3</td>
</tr>
<tr>
<td>Thickness, μm</td>
<td>4.2 ± 0.1</td>
<td>4.3 ± 0.1</td>
</tr>
<tr>
<td>Internal diameter-to-thickness index</td>
<td>1.75 ± 0.5</td>
<td>1.65 ± 0.5</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 12 control rats and 15 anemic rats.
the case of the in vitro hearts perfused with a very low-hematocrit solution, where the capillary hematocrit greatly exceeds that in the perfusate. This cannot be explained by the presence of trapped RBCs in the capillaries of the isolated perfused hearts: perfusion with a near-to-normal hematocrit results in values of capillary spacing and the percentage of RBCs touching (0 RBC spacing) that are close to values observed under in vivo conditions.

Our results clearly indicate compensatory mechanisms at the level of the coronary microcirculation, which are more pronounced in acute hemodilution and particularly in isolated hearts perfused with low-hematocrit solution. Capillary linear hematocrit depends on the hematocrit in the supplying blood, on the percentage of capillary segments perfused with plasma only or with an occasional RBC as well as on the rate of flow in these capillaries, and, finally, on the velocity of the RBCs in the remaining capillary segments. In our experimental situation, we cannot readily identify the relative contribution of these individual determinants. An important role is probably played by decreased viscosity of the blood as well as by the increase in the number of capillaries perfused with plasma only, which contributes to higher plasma flow compared with the RBC flow.

Several theoretical studies (5, 8, 9) demonstrated that as the distance between RBCs increases, a constant \( O_2 \) flux cannot be maintained. A critical value for this spacing is, of course, variable and depends on tissue \( O_2 \) consumption, RBC flux, etc. Moreover, tissue oxygenation at low capillary hematocrit was found to be highly sensitive to capillary spacing (32). RBC distribution within the microvascular bed would therefore influence the diffusion capacity in both the alveolar capillaries (10) as well as the peripheral circulation (13). Our finding of a smaller decrease in the RBC distribution within the coronary microvascular bed compared with the systemic hematocrit may be considered as an adaptive reaction to acute or chronic hemodilution. The decrease was less pronounced in acute experiments, which would exclude significant contribution of morphological adjustments. A detailed mechanistic explanation for these adaptations remains to be elucidated.

In conclusion, we have found evidence of angiogenesis in cardiomegaly induced by chronic anemia. Compensatory microvascular growth was accompanied by an advantageous regulation of RBC spacing within these vessels. An attempt toward preservation of a normal RBC spacing pattern was even more pronounced during acute hemodilution in vivo and in isolated perfused hearts.

The authors thank Barbara Hebert and Ching Kuo for excellent technical assistance.

This study was supported by grants from Medical Research Council of Canada and Ontario Heart and Stroke Foundation.

### Table 5. RBCs within capillaries in vivo and in isolated perfused hearts

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Chronic Anemia</th>
<th>Acute Anemia</th>
<th>High Hct</th>
<th>Medium Hct</th>
<th>Low Hct</th>
</tr>
</thead>
<tbody>
<tr>
<td>( n )</td>
<td>16</td>
<td>18</td>
<td>16</td>
<td>15</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>Systemic Hct, %</td>
<td>45.1 ± 0.5</td>
<td>24.9 ± 0.6f</td>
<td>24.3 ± 0.7f</td>
<td>39.4 ± 1.5</td>
<td>29.4 ± 1.3b</td>
<td>14.2 ± 2.1ac</td>
</tr>
<tr>
<td>Capillary Hct, %</td>
<td>28.5 ± 0.8</td>
<td>18.4 ± 0.7fes</td>
<td>22.1 ± 1.4f</td>
<td>37.1 ± 1.3</td>
<td>25.7 ± 1.3a</td>
<td>23.6 ± 1.8ase</td>
</tr>
<tr>
<td>RBC spacing, μm</td>
<td>12.1 ± 0.5</td>
<td>18.6 ± 0.8f</td>
<td>17.2 ± 1.2f</td>
<td>10.4 ± 0.5</td>
<td>15.0 ± 0.7f</td>
<td>18.2 ± 0.9sfad</td>
</tr>
<tr>
<td>%RBC spacing = 0</td>
<td>12.3 ± 1.1</td>
<td>7.0 ± 0.6f</td>
<td>7.5 ± 1.1f</td>
<td>11.3 ± 1.1</td>
<td>8.2 ± 0.9f</td>
<td>4.6 ± 0.6f</td>
</tr>
<tr>
<td>%RBC spacing &gt; 40 μm</td>
<td>5.9 ± 0.6</td>
<td>11.9 ± 1.0f</td>
<td>10.8 ± 1.6f</td>
<td>3.6 ± 0.4</td>
<td>8.3 ± 0.9f</td>
<td>10.7 ± 1.0fae</td>
</tr>
</tbody>
</table>

Values are means ± SE, \( n \) = no. of hearts. RBCs, red blood cells; Hct, hematocrit. Significant differences from high Hct: \( aP \leq 0.001 \) and \( bP < 0.01 \); from medium Hct: \( cP < 0.001 \), \( dP < 0.001 \), and \( eP < 0.05 \); from control: \( fP < 0.001 \); and from acute: \( gP < 0.02 \).
REFERENCES


