Regional Myocardial Capillary Erythrocyte Transit Time in the Normal Resting Heart

Michael F. Allard, Craig T. Kamimura, Dean R. English, Sarah L. Henning, and Barry R. Wiggs

A major determinant of oxygen transport to the myocardium is the time spent by the erythrocytes (red blood cells [RBCs]) traversing the microcirculation. Although it has been shown that the myocardium has regional differences in blood volume, blood flow, metabolism, and sensitivity to ischemic injury, the regional distribution of RBC transit times through the myocardial capillaries has not been previously measured. The present study was designed to measure the regional myocardial capillary RBC transit time by a new technique to determine whether there are regional differences in the capillary RBC transit time in the normal resting heart. Anesthetized open-chest male New Zealand White rabbits (3.0–3.7 kg, n=8) were studied. Regional myocardial blood volume was determined using chromium-51-labeled RBCs, and regional blood flow was measured using a reference flow technique and a left atrial injection of 15-μm–radiolabeled (gadolinium-153, 10–20 μCi) microspheres. Capillary blood volume was determined by multiplying the regional blood volume by the histologically determined fraction of the total blood volume that was in the capillaries. Capillary RBC transit time was calculated as the quotient of capillary blood volume and blood flow. The myocardial capillary blood volume was the same in the endocardium and the epicardium (4.67±0.67 ml/100 g for endocardium versus 4.52±0.70 ml/100 g for epicardium, p=NS), whereas myocardial blood flow tended to be greater in the endocardium (6.09±0.73 ml/sec per 100 g for endocardium versus 5.47±0.75 ml/sec per 100 g for epicardium), although this was not statistically significant. Myocardial capillary RBC transit times ranged from 0.22 to 2.58 seconds with a mean of 0.89±0.13 and 0.85±0.13 seconds and a median of 0.72 and 0.70 seconds in the epicardium and endocardium, respectively. We conclude that there are no regional differences in the myocardial capillary RBC transit time in the normal heart at rest. (Circulation Research 1993;72:187–193)

KEY WORDS • erythrocyte transit time • oxygen transport • myocardium

Increases in myocardial oxygen consumption in response to modest levels of work are generally accomplished by corresponding increases in coronary blood flow.1,2 At more extreme levels of cardiac work, increases in coronary flow are insufficient, and increased extraction of O2 from the blood may contribute up to 40% of the increased myocardial oxygen consumption.3 A major determinant of the transport of O2 from blood to tissue is the time spent by erythrocytes (red blood cells [RBCs]) passing through the capillaries, the capillary RBC transit time.3–9 The capillary transit time in a given region of myocardium is dependent on the blood volume and blood flow in that region, both of which may vary significantly.1,10,11 If the capillary RBC transit time also shows regional differences, it might be an important factor contributing to the well-known regional variability in myocardial susceptibility to ischemia.12 Although the RBC transit time in cardiac muscle has been previously determined by others,4,7,9,11 no study has yet specifically measured the myocardial RBC transit time in the capillaries of different regions of the heart. The present study was designed to measure the regional myocardial capillary RBC transit time by a new technique to determine if there are regional differences in the capillary RBC transit time in the normal resting heart.

Materials and Methods

Animal Preparation

Male New Zealand White Rabbits (3.0–3.7 kg, n=8) were anesthetized with intravenous urethane (1 g/kg) and chloralose (100 mg/kg).13 A midline tracheotomy was performed, and the animals were mechanically ventilated. Catheters were placed in the right carotid and left femoral arteries for measurement of systemic blood pressure and the collection of the reference flow sample and in an ear vein for the administration of drugs and fluids. The animals were paralyzed by a slow bolus infusion of succinylcholine (1 mg/kg i.v.) followed by a continuous infusion (1 mg/kg/hr) into the ear vein. A midline thoracotomy was then performed, and a catheter was placed in the left atrial appendage and secured by a suture. The small animal ventilator settings and the oxygen content of the inspired air were adjusted to maintain Pao2 between 80 and 140 mm Hg and Paco2 between 24 and 28 mm Hg. Periodic positive end-expiratory pressure (10 cm H2O) was administered throughout the experiment to prevent atelectasis. Nor-
saline was infused (1 ml/min) into the ear vein, and arterial blood pressure was monitored throughout.

**Experimental Protocol**

The rabbits were allowed to stabilize for 30 minutes after completion of the surgery. Myocardial blood flow was measured by the reference flow technique by means of a left atrial injection of 15-μm microspheres (gadolinium-153, 10–20 μCi). The microspheres were injected in 1 ml saline over 15–20 seconds followed by a 5-ml saline flush. The microspheres were vigorously vortexed for 2 minutes immediately after injection. Approximately 700,000–1,000,000 microspheres were injected to obtain greater than 400 microspheres in each myocardial sample to ensure reliable flow measurements. The reference flow was collected at a rate of 4 ml/min into preweighed tubes rotating on a fraction collector every 10 seconds. Collection began 15 seconds before the injection of the microspheres and continued for 2.5 minutes. This technique, using multiple collection tubes, allowed evaluation of the fractions to ensure complete collection of microspheres in the reference sample. During injection of the microspheres, the recorder paper speed was increased to detect abnormalities of heart rhythm. Heparinized, washed RBCs obtained from a donor rabbit and resuspended in normal saline were infused into the ear vein at a rate of 4 ml/min, concurrent with the withdrawal of the reference sample, to prevent a significant decrease in blood pressure during withdrawal. At the completion of the blood flow measurement, the rabbits were given RBCs obtained from a donor rabbit and labeled with chromium-51 (500 μCi). After allowing 5 minutes for the labeled RBCs to circulate, a reference sample of arterial blood was taken, and the base of the heart was rapidly clamped to maintain the myocardial blood volume present during life. An ice and saline slush was immediately poured into the thoracic cavity to arrest the heart. Cessation of visible contractile activity usually occurred within 30 seconds. The heart was then removed from the thoracic cavity, a tie was secured around the base of the heart, and the heart was placed in 10% formalin. Both kidneys were removed in entirety, sectioned, placed in preweighed scintillation vials, and weighed. They were subsequently counted with the myocardial samples to evaluate the adequacy of mixing of microspheres.

After fixation in formalin, the heart was processed in a standardized manner. The heart was transversely sectioned parallel to the atrioventricular groove into five slices (Figure 1). The right ventricular free wall was removed from the left ventricle plus septum of each slice. The middle three slices of the left ventricle plus septum were divided into anterior, lateral, posterior, and septal regions. Each region was then divided into an inner portion (endocardium [ENDO]) and an outer portion (epicardium [EPI]) or into right and left portions in the case of the septal pieces. The basal and apical slices of the left ventricle plus septum were each cut in half in the sagittal plane. Myocardial samples were then placed individually in preweighed scintillation vials and weighed. Five milliliters of 10% formalin was added to each vial before gamma counting.

The gamma-emitting radionuclides were counted in a gamma scintillation counter (model 8000, Beckman Instruments, Inc., Fullerton, Calif.). The gamma counter was linked to an IBM computer, with windows selected to maximize counts for each radionuclide while minimizing the spillover into other channels. Appropriate corrections were made for background, decay, and overlap.

**Histological Determination of the Volume Fraction of Myocardial Capillaries**

The myocardial blood volume determined by isotopic methods alone yields a blood volume measurement that is distributed among large, intermediate, and small blood vessels. To determine the volume of blood in capillaries in a given myocardial sample, the proportion of blood vessel volume that is capillary was assessed histologically in each myocardial sample. A representative section of myocardium from each ENDO and EPI region was embedded in glycol methacrylate, sectioned at 2 μm, and stained with methenamine-silver. The methenamine-silver stain highlights the basement membrane zone (Figure 2) and thus allows easier identification and quantification of myocardial blood vessels. The volume fraction of myocardial blood vessels was determined by point counting using a multilevel technique similar to that described by Cruz-Orive and Weibel. The point counting, using a 100-point grid, was carried out at three levels of magnification: low (×40), to distinguish between fat large vessels (>100-μm diameter) and “myocardium”; medium (×100), to distinguish between intermediate vessels (10-<100-μm diameter) and “myocardium”; and high (×400), to distinguish between capillaries (<10-μm diameter) and myocardium. The entire myocardium was counted at the low magnification; random fields were used at the medium and high magnifications. A total of 1,500 points were counted per section at these two higher magnifications, yielding an approximate relative standard error of less than 10%. The proportion of
myocardial blood vessel volume fraction occupied by capillaries and intermediate and large vessels was calculated as the quotient of the area of the particular vessel size in question and the sum of the area occupied by all vessels.\textsuperscript{19}

**Calculations**

The myocardial blood volume (V\textsubscript{m}) was calculated from the \textsuperscript{5}\textsuperscript{1}Cr activity in each piece, the specific activity of \textsuperscript{5}\textsuperscript{1}Cr in the arterial blood reference sample, and the density of blood (1.06 g/ml). The myocardial blood flow (Q\textsubscript{m}) was calculated using the formula Q\textsubscript{m} = Q\textsubscript{r} \times C\textsubscript{m}/C\textsubscript{r}, where Q\textsubscript{r} is the reference flow (in milliliters per minute), C\textsubscript{m} is counts per minute of \textsuperscript{15}\textsuperscript{Gd in the myocardium, and C\textsubscript{r} is counts per minute of \textsuperscript{15}\textsuperscript{Gd in the reference sample. The regional myocardial RBC transit time was determined as the quotient of blood volume and flow (V\textsubscript{m}/Q\textsubscript{m}).\textsuperscript{19}

The volume of blood within the different vessel size categories was determined in each myocardial piece by multiplying the proportions of the different vessel sizes and the total blood volume in that piece, e.g., P\textsubscript{c} \times V\textsubscript{m} = V\textsubscript{c}, where P\textsubscript{c} is the proportion of myocardial blood vessel fraction occupied by capillaries and V\textsubscript{c} is capillary blood volume. The capillary RBC transit time was then calculated for each myocardial sample using the ratio V\textsubscript{c}/Q\textsubscript{m}.

In addition, histological techniques were also used to determine the volume of blood in each myocardial sample. The histologically derived blood volume in each myocardial sample was determined from the volume fraction of the myocardium that was blood vessel and the volume of each myocardial sample, which was calculated from the sample weight and tissue density (1.06 g/ml).

All values were normalized to the blood-free myocardial sample weight and were expressed per 100 g myocardium.

**Statistical Analysis**

All data are expressed as mean±SEM except where otherwise noted. Paired t tests corrected for multiple comparisons using a sequentially rejective Bonferroni procedure\textsuperscript{20} were used to test for differences between regions.

**Results**

**Hemodynamics and Arterial Blood Gases**

The open-chest anesthetized rabbits had mean systolic and diastolic blood pressures of 89±7 and 61±4 mm Hg, respectively, with a mean heart rate of 259±12 sec\textsuperscript{-1}. Arterial blood gas analysis showed a mean Pa\textsubscript{o} of 113±7 mm Hg and a mean Pac\textsubscript{o} of 26±1 mm Hg. Cardiac output was determined in two additional open-chest anesthetized rabbits by indicator dilution techniques.\textsuperscript{16} The cardiac outputs of these two rabbits were 581 and 511 ml/min, which were similar to results obtained previously in our laboratory in a closed-chest anesthetized rabbit preparation.\textsuperscript{16}

**Myocardial Blood Volume and Blood Flow**

The mean left ventricular myocardial blood volume determined with \textsuperscript{51}Cr RBCs was 6.30±1.08 ml/100 g. The myocardial blood volume tended to be greater in the EPI compared with the ENDO region of the myocardium (7.01±1.31 versus 5.59±0.93 ml/100 g, p=NS) (Table 1).

Myocardial blood flow was 5.78±0.73 ml/sec per 100 g, which accounted for approximately 3–5% of the

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Q, myocardial blood flow; V, myocardial blood volume; TT\textsubscript{a}, myocardial erythrocyte transit time; TT\textsubscript{c}, myocardial capillary erythrocyte transit time; EPI, epicardial myocardium; ENDO, endocardial myocardium. Values are mean±SEM.

\textsuperscript{*}p<0.05 vs. EPI value (n=8).
measured cardiac output in this animal preparation. There was a trend toward slightly greater blood flow to the ENDO compared with the EPI (6.09±0.73 versus 5.47±0.75 ml/sec per 100 g, p=NS) (Table 1). In addition, both the myocardial blood volume and blood flow had heterogeneous distributions. The coefficient of variation or relative dispersion, defined as the quotient of the standard deviation and the mean, provides a measure of the heterogeneity. The myocardial blood volume and blood flow showed relative dispersions among animals of 0.47 and 0.36, respectively.

The histologically derived mean myocardial blood volume was 11.71 ml/100 g, which was almost twice as great as that measured with 51Cr RBCs. The degree of regional heterogeneity tended to be less, particularly in the ENDO, than the corresponding values for blood volume measured with radiolabeled RBCs. Relative dispersions among animals were 0.20 and 0.09 in the EPI and ENDO, respectively.

**Myocardial Capillary Blood Volume**

Histological evaluation of the myocardium showed that the EPI had a significantly greater proportion of large vessels than the ENDO (22.97±2.78% for EPI versus 4.79±1.19% for ENDO, p<0.05), whereas the ENDO had a greater proportion of capillaries (86.13±2.36% for ENDO versus 68.85±2.80% for EPI, p<0.05) (Figure 3A). There were no regional differences in the proportion of intermediate-sized blood vessels. The volume of blood in myocardial capillaries was not different in the ENDO compared with the EPI, whereas the volume in large vessels was greater in the EPI (Figure 3B). The blood volume in intermediate vessels showed no regional differences.

**Capillary RBC Transit Time**

The myocardial capillary RBC transit times from all hearts ranged from 0.22 to 2.58 seconds with medians of 0.72 and 0.70 seconds and means of 0.89±0.13 and 0.83±0.13 seconds in the EPI and the ENDO, respectively (Figure 4, Table 1). The relative dispersion of capillary RBC transit times among animals was 0.45 in the EPI and 0.47 in the ENDO. The mean capillary transit times are in distinct contrast to the mean myocardial transit times, which were determined without the contribution of blood in large vessels taken into account; the mean myocardial transit time in the EPI (1.38±0.26 seconds) was significantly greater than that in the ENDO (0.99±0.19 seconds, p<0.05).

**Discussion**

Myocardial oxygen consumption and blood flow are closely matched at low to intermediate workloads, and the extraction of the oxygen from the blood by the heart remains relatively constant. Higher workloads, however, such as those seen with maximal exercise, are associated with a substantial increase in the extraction of oxygen that may account for up to 40% of the increased oxygen consumption. These findings suggest that alterations take place within the microcirculation and result in the near complete extraction of oxygen delivered to the myocardium. Parameters of the microcirculation whose alteration may contribute to the increased extraction of oxygen include the functional capillary surface area, the oxygen content and hematocrit, the oxygen concentration gradient, the time for oxygen release, and the capillary RBC transit time. The time required by RBCs to pass through the capillaries has been shown to be a major determinant of the transport of oxygen in striated muscle. The RBC transit time in any vascular bed is dependent on the blood flow to that bed and the volume of blood through which it passes, such that, if blood flow increased without an accompanying increase in blood volume, the transit time would decrease. The myocardium has been shown to have significant regional differences in both blood flow and vascularity. Under resting conditions in normal hearts, the blood flow, measured by the reference flow technique with radiolabeled microspheres, and the microvascular blood volume tend to be greater in the inner myocardium. Since blood volume and blood flow are the major determinants of the RBC transit time, discordant regional alterations in either may result in significant regional differences in the capillary RBC transit time. If regional differences in the RBC transit time do occur, these differences may contribute to the regional variability in sensitivity of the myocardium to ischemia and ischemic injury. It is likely that these regional disparities, if present, will be exaggerated and become significant only during hemodynamic stress in normal and, particularly, in diseased hearts, such as those with pressure-overload hypertro-
Myocardial Capillary Erythrocyte Transit Time

phy. The myocardial RBC transit time has been previously determined in other studies by means of indicator dilution techniques,7,9,25 by calculations based on estimated capillary pathway length and measured erythrocyte velocities in myocardial capillaries,4 and by techniques similar to those described in the present study.11 No study to date, however, has specifically determined the regional distribution of myocardial capillary RBC transit times. The present study was designed to measure the regional myocardial capillary RBC transit time to determine if there are regional differences in capillary RBC transit time in the myocardium of normal resting hearts.

The myocardial blood flow in this preparation accounted for approximately 3–5% of the cardiac output and showed the well-described trend for greater blood flow toward the inner myocardium1 (Table 1). Blood flow to the myocardium was also found to be regionally heterogeneous. The relative dispersion of blood flows among animals was 0.35, a value comparable to that previously reported in anesthetized rabbits11 and conscious baboons.26

Myocardial blood volumes reported in the literature vary substantially.11,22,24,27 Our results compare favorably with blood volumes reported in the dog24 and the rat.22,27 The myocardial blood volumes in the present study likely represent an average of systolic and diastolic blood volumes. The blood volumes are intermediate to those reported in the rat, in which the effects of barium-induced contracture and potassium chloride–induced arrest on intramyocardial blood volume were determined.27 The barium-induced contracture was felt to recapitulate systole, whereas potassium chloride–induced arrest was felt to reproduce diastole. A recent study by Gonzalez and Bassingthwaighte11 reported blood volumes in rabbit myocardium higher than those in the present study. The differences between these two studies may be partially explained by differences in anesthesia, possible anesthesia-related changes in hemodynamics, and techniques of measurement. In addition, the calculation of blood volume in the present study did not take into account the decreased hematocrit of blood in the myocardium compared with systemic vessels.11 As a result, the myocardial blood volume measurements slightly underestimate the true myocardial blood volume. However, the degree of underestimation, assuming a myocardial hematocrit of approximately 75% of large vessel hematocrit,11 is insufficient to entirely explain the differences between the data of Gonzalez and Bassingthwaighte and our own.

Histological analysis of the myocardium (Figure 3A) showed that capillaries represented 86.1±2.4% of all vessels in the ENDO and 68.9±2.8% of all vessels in the EPI (p<0.05). These values compare favorably with those reported by others, who found that 85–86% of the myocardial vascular volume was located in the capillaries28 or the terminal vascular bed22 in normal dog hearts. The myocardial capillary blood volume, determined from the proportion of all blood vessels that are capillaries and the measured blood volume in each myocardial sample, showed no differences between the EPI and ENDO (Figure 3B). The tendency for the mean myocardial blood volumes to be greater in the EPI region (Table 1) can be accounted for by the preponderance of larger coronary vessels in this location (Figure 3).

The techniques used in the histological analysis of the myocardium measure the entire anatomic compartment of myocardial blood vessels29 and, therefore, the theoretical maximal attainable myocardial blood volume. The technique does not, however, account for the possibility that all vessels in that compartment may not be perfused under resting conditions.29 The myocardial blood volume measured by 51Cr-labeled erythrocytes was 56.4±7.6% of the histologically derived theoretical maximal value. These findings are consistent with data reported by others in rabbits in which approximately 50–60% of myocardial capillaries and arterioles were perfused under resting conditions.29 The myocardial blood volume determined with 51Cr RBCs also showed greater heterogeneity than that derived from histological methods. The increased heterogeneity of blood volume when measured with labeled erythrocytes is likely a reflection of different degrees of perfusion of the various myocardial regions. The proportion of myocardial capillaries and arterioles perfused has been shown to be similar under resting conditions in hearts from anesthetized open-chest rabbits.29 Since the heterogeneity of perfusion in that study29 was likely com-

comparable to that in our preparation, it was assumed that the proportion of vessels perfused in the present study was not different among the three different categories of vessel size. Thus, the proportion of different-sized vessels determined histologically was used to calculate the corresponding blood volumes. However, subsequent studies in which hearts are examined under nonresting conditions must specifically evaluate the proportions of the various-sized vessels perfused because, according to previous studies, the distribution of perfusion throughout the myocardium may not be uniform during hemodynamic stress.30,31

The myocardial capillary RBC transit time was determined in different regions of the heart for the first time in the present study by measuring regional capillary blood volume and blood flow. These parameters showed substantial variability among as well as within individual hearts (Figure 4, Table 2). Although the heterogeneity varied up to fivefold among hearts, within a given heart the heterogeneity was, interestingly, generally comparable between ENDO and EPI regions (Figure 4). By combining the RBC capillary transit time data from all hearts (Figure 5), a representation of all possible transit times under the conditions of study may be obtained. The myocardial capillary transit times from all hearts ranged from 0.22 to 2.58 seconds (Figure 5) with medians of 0.72 and 0.70 seconds and means of 0.89 ± 0.13 and 0.83 ± 0.13 seconds in the EPI and the ENDO, respectively. The relative dispersion of capillary transit times among the different animals was comparable to that of myocardial blood volume and flow and measured 0.45 in the EPI and 0.47 in the ENDO. Within individual hearts, the relative dispersion of capillary transit times ranged from 0.13 to 0.33 in the ENDO and from 0.20 to 0.43 in EPI (Figure 4). Although the mean myocardial capillary RBC transit times measured in the present study (Table 1) are somewhat shorter than those reported from studies using indicator dilution techniques,25 they are comparable to RBC transit times estimated by others to occur in the myocardium under resting conditions.3,6 Of particular interest with regard to oxygen transport was the finding that the vast majority of myocardial capillary RBC transit times were greater than the estimated minimum time for oxygen to be released from the erythrocytes to the resting myocardium.6 Although the data are not strictly comparable, this finding suggests that the majority of capillary RBC transit times are sufficiently long to allow for adequate O2 release in the normal resting heart and that O2 transport to the myocardium under these conditions is not likely limited by the capillary RBC transit time.

The techniques used in the present study to determine the capillary RBC transit time in small myocardial samples and composite regions (ENDO and EPI) of the heart do have some limitations. The capillary RBC transit times in the myocardial samples represent mean values of transit times through the individual capillaries in a given piece of myocardium. It is these individual

![Figure 5](http://circres.ahajournals.org/lookup/figure/1993/01/192-CR-192-01543.jpg)
RBC transit times through the myocardial microcirculation and, in particular, their heterogeneity that are the values most relevant to oxygen transport in the myocardium. The heterogeneity of RBC transit times observed at the level of the myocardial samples in the present study does not, however, necessarily reflect that of the transit time through individual capillaries. Currently, there are no techniques available that can directly measure individual myocardial capillary transit times. However, the distribution of capillary transit times has been determined in skeletal muscle either directly, by measurement of the transit time of fluorescent-labeled RBCs across a capillary network, or indirectly, by calculations based on an estimated distribution of capillary path lengths and velocity measurements in superficially located microvessels. Although velocity measurements of RBCs in epicardial capillaries are available, the distribution of the lengths of the individual pathways taken by the RBCs through the myocardial microcirculation is not yet known. Perhaps, in the future, computer models, based on detailed morphological and hemodynamic analyses of the myocardial microcirculation, will shed light on the important RBC transit time distributions through the individual myocardial capillaries.

In summary, the regional myocardial capillary RBC transit time, a major determinant of oxygen transport to the myocardium, was measured for the first time by a new technique. We demonstrate that there are no significant regional differences in myocardial capillary RBC transit time under resting conditions. Whether significant alterations occur in the myocardial capillary RBC transit time in normal and diseased hearts with hemodynamic stress and how these possible alterations may affect O₂ transport remain to be determined.

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