Effects of red blood cell aggregation on myocardial hematocrit gradient using two approaches to increase aggregation

Ozlem Yalcin,1 Funda Aydin,2 Pinar Ulker,1 Mehmet Uyuklu,1 Firat Gungor,2 Jonathan K. Armstrong,3 Herbert J. Meiselman,3 and Oguz K. Baskurt1

Departments of 1Physiology and 2Nuclear Medicine, Akdeniz University Faculty of Medicine, Antalya, Turkey; and 3Department of Physiology and Biophysics, Keck School of Medicine, University of Southern California, Los Angeles, California

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Yalcin, Ozlem, Funda Aydin, Pinar Ulker, Mehmet Uyuklu, Firat Gungor, Jonathan K. Armstrong, Herbert J. Meiselman, and Oguz K. Baskurt. Effects of red blood cell aggregation on myocardial hematocrit gradient using two approaches to increase aggregation. Am J Physiol Heart Circ Physiol 290: H765–H771, 2006. First published September 19, 2005; doi:10.1152/ajpheart.00756.2005.—The normal transmyocardial tissue hematocrit distribution (i.e., subepicardial greater than subendocardial) is known to be affected by red blood cell (RBC) aggregation. Prior studies employing the use of infused large macromolecules to increase erythrocyte aggregation are complicated by both increased plasma viscosity and dilution of plasma. Using a new technique to specifically alter the aggregation behavior by covalent attachment of Pluronic F-98 to the surface of the RBC, we have determined the effects of only enhanced aggregation (i.e., Pluronic F-98-coated RBCs) versus enhanced aggregation with increased plasma viscosity (i.e., an addition of 500 kDa dextran) on myocardial tissue hematocrit in rapidly frozen guinea pig hearts. Although both approaches equally increased aggregation, tissue hematocrit profiles differed markedly: 1) when Pluronic F-98-coated cells were used, the normal transmyocardial gradient was abolished, and 2) when dextran was added, the hematocrit remained at subepicardial levels for about one-half the thickness of the myocardium and then rapidly decreased to the control level in the subendocardial layer. Our results indicate that myocardial hematocrit profiles are sensitive to both RBC aggregation and to changes of plasma viscosity associated with increased RBC aggregation. Furthermore, they suggest the need for additional studies to explore the mechanisms affecting RBC distribution in three-dimensional vascular beds.

myocardium; plasma viscosity; Pluronic F-98; dextran

THE DIFFERENCE BETWEEN large blood vessels and microvascular hematocrit values was first described by Fahraeus (12) and later confirmed by other experimental studies (17, 18, 21, 33). The mean hematocrit value for blood in the vasculature of a given tissue, usually referred to as tissue hematocrit, tends to be significantly lower than the hematocrit value in arterial or venous blood (28). Therefore, the venous hematocrit value that is routinely measured for clinical purposes is actually an incorrect estimate of the mean hematocrit value of the total circulating blood volume. Furthermore, the reduced tissue hematocrit contributes to the reduced apparent viscosity of blood under in vivo flow conditions (34) and affects oxygen transfer to the tissues being perfused (17).

The lower mean hematocrit value for a given tissue generally reflects the significant reduction of hematocrit as the blood approaches the microcirculation. The mechanisms that underlie this reduction are multiple and interrelated and are related to the nonuniform distribution of red blood cells (RBCs) across the vessel diameter. This nonuniform distribution is due to two phenomena. First, because RBCs have a finite size, their local concentration near the wall is reduced, i.e., a wall exclusion effect, resulting in a statistical cell-poor layer having a width of about one-half of the cell diameter or less. Second, during flow, there is a tendency for RBCs to accumulate in the central region of vessels, a process known as axial migration (19, 28). The nonuniform distribution of cells leads to two important phenomena: plasma skimming and the Fahraeus effect (19, 28). Plasma skimming, i.e., a greater plasma flow in the branches, is due to the feeding of side branches from the cell-poor plasma layer adjacent to the vessel wall. The Fahraeus effect is the lowered hematocrit for blood within a given vessel segment compared with the hematocrit of blood that is discharged from that vessel segment and is due to the higher flow velocity of RBCs in the central flow zone relative to the plasma-rich fluid in the marginal zone (19, 28). In addition to these mechanisms, there is flow partitioning at vascular bifurcations, resulting in a preferential accumulation of RBCs in the branch with the higher flow rate. This phenomenon, termed “network Fahraeus effect,” is effective in generating a Fahraeus effect at the circulatory network level rather than for individual vessels (16, 25).

The factors that determine the difference between the mean hematocrit value for a given tissue and systemic hematocrit are not well documented. Axial migration of RBCs during flow can be affected by various factors (10, 19), including the rheological properties of the cells and RBC aggregation. Fahraeus (12) was the first to show that greater enhancement of RBC axial migration in experiments using horse blood versus human blood. Employing vertical glass tubes, Cokelet and Goldsmith (10) demonstrated that enhanced RBC aggregation may lead to a thicker cell-poor layer at the wall. These findings suggest that the reduction of tissue hematocrit might be dependent on the degree of RBC aggregation. Detailed studies of tissue hematocrit in myocardium have demonstrated spatial differences and the effects of cell rigidity and aggregation. First, Vicaut and Levy (33) demonstrated that tissue hematocrit values differ between subepicardial and subendocardial layers of the left ventricular myocardium and that there is a linear gradient between the two layers in hearts arrested during diastole by rapid freezing. Second, enhancing RBC
aggregation by infusion of fibrinogen results in altered tissue hematocrit values in rat myocardium (2). Finally, reduced RBC deformability alters the tissue hematocrit gradient that exists in normal rat myocardium (3). Although variation of blood vessel morphometric parameters within the myocardium could lead to the observed gradients, differences in blood vessel sizes required to explain the gradients do not exist (33). Rather, it has been suggested that variations of discharge hematocrit at different myocardial layers are responsible for the gradient (33).

Although the effects of RBC aggregation on myocardial hematocrit gradient have been reported, increases of aggregation were achieved by adding fibrinogen to the plasma used to suspend the RBCs (2). The addition of fibrinogen alters the physicochemical characteristics of the plasma and increases plasma viscosity. Such changes may interfere with vascular control mechanisms, vessel size, and the distribution of RBCs. However, a new technique employing covalent attachment of Pluronic F-98 to the RBC surface has been developed, which allows the enhancing of RBC aggregation without the need for the infusion of a macromolecular solution and, hence, without an alteration of the suspending phase properties, e.g., plasma protein concentration and plasma viscosity. The present study was thus designed to compare the effects on myocardial hematocrit gradient of enhanced RBC aggregation, as induced either by the addition of a high-molecular-weight polymer to plasma or by Pluronic F-98 attachment to the RBC surface.

MATERIALS AND METHODS

Animals and groups. Adult guinea pigs of both sexes weighing 400–500 g were used in the experiments. The animals were randomly divided into control, aggregation I, and aggregation II groups. Another group of animals was used as blood donors for the preparation of RBC suspensions to be used in exchange transfusions. Blood from donor animals was obtained by cardiac puncture under light ether anesthesia, anticoagulated with EDTA (1.5 mg/ml), and handled as described below. The experimental protocol was approved by the Animal Care and Use Committee of Akdeniz University and was in accordance with the Declaration of Helsinki and the International Association for the Study of Pain guidelines.

Preparation. Guinea pigs were anesthetized with urethane (1 g/kg ip), tracheotomies were performed, and animals were ventilated with ambient air at a rate of 70 breaths/min with a tidal volume of 1 ml. The left carotid artery was catheterized using a polyethylene catheter (20 gauge). The catheter was positioned at the level of the ascending aorta and connected to a pressure transducer. Arterial blood pressure was recorded throughout the experiment and during the freezing of the heart. The left jugular vein was also catheterized and used for exchange transfusions together with the carotid artery. These catheters were also used for injection of labeled blood samples and blood sampling.

Exchange transfusions. During the exchange transfusions in all groups, ~40% of the total blood volume of animals was exchanged. The total blood volume of animals was estimated as 8% of their body weight. The control group received an exchange transfusion with normal hematocrit-matched guinea pig blood before the determination of myocardial hematocrit gradient. In the aggregation I group, RBCs coated with Pluronic F-98 to enhance aggregation were suspended in unaltered plasma and used for exchange transfusion. In the aggregation II group, the exchange transfusion was performed by using normal guinea pig RBCs suspended in plasma containing 1 g/dl Dextran 500 (500 kDa, Sigma Chemical, St. Louis, MO). Although RBC aggregation was enhanced in both aggregation groups, the means used to increase RBC aggregation, and hence the final plasma composition and viscosity, were different (1). The procedure (see Determination of myocardial hematocrit gradient) was started 5 min after the completion of the exchange transfusion.

Determination of myocardial hematocrit gradient. Tissue hematocrit gradient in the left ventricular myocardium was estimated by determining the activity of two different radionuclides, which labeled plasma and RBCs, as described by Vicaut and Levy (33). RBCs were labeled in vitro with 99mTc and 125I-labeled albumin (Amersham) was used to trace plasma. 125I-Labeled albumin was purified by using an ultracentrifuge with a molecular-mass cutoff of 50 kDa (Microsep) to eliminate any unbound tracer. 99mTc-labeled RBCs (0.2 ml packed cells) and 0.15 ml 125I-labeled albumin solution (20 mg/ml albumin, 1 μCi) were suspended in 0.65 ml of saline and injected through the femoral vein. After 5 min, 1 ml of blood was sampled for the determination of 99mTc and 125I activities, systemic hematocrit, and hemorheological parameters (see Microscopic and rheological studies).

After blood sampling was completed, a rapid midsternal thoracotomy was performed, and a plastic cylinder was immediately positioned around the heart. This cylinder was rapidly filled with liquid nitrogen, thus quickly freezing the heart. The frozen heart was then excised and kept at −20°C. A 2 × 3- × 5-mm tissue block with the long axis perpendicular to the left ventricular wall was cut and mounted on a cryostat disk, with the disk surface parallel to the left ventricular wall. With the use of a cryotome (Shandon Cryotome AS620, Cheshire, UK), each tissue block was sectioned through the thickness of the left ventricular wall, from epicardium to endocardium, to obtain 100-μm-thick myocardial slices. Sectioning was continued until the left ventricular cavity, clearly distinguished by the color of blood, was approached. Any myocardial tissue block with an unusual geometry observed during slicing (e.g., slices partially covered with blood) was excluded from the study. Each slice was numbered according to its depth in the tissue, with the epicardial layer corresponding to 0 and the layer closest to endocardium corresponding to 1. Slices between these two were represented by values between 0 and 1, depending on the depth and the number of 100-μm-thick slices. A transverse section of the heart was also used to measure the thickness of the left ventricular wall and to determine whether the heart was arrested during systole or diastole. Only the hearts arrested during diastole were used for data analysis.

Radionuclide (99mTc and 125I) activities in the systemic blood specimens and the myocardial tissue slices were measured by a gamma counter (Atomlab 950, Biodex Medical Systems, New York, NY). Systemic hematocrit was determined by the microcapillary method (12,000 g, 5 min). Tissue hematocrit was estimated in each myocardial slice according to Vicaut and Levy (33). Briefly, for each tracer and each blood sample, a constant K between the radionuclide activity and the volume of the labeled compartment was calculated by using the measurements obtained for the systemic blood specimens: $K_{\text{sys}} = V_{\text{blood}} \times \text{Hct}_{99mTc}$ counts/min; $K_{125I} = [V_{\text{blood}} \times (1 - \text{Hct})]^{1/125I}$ counts/min, where $V_{\text{blood}}$ is the volume of blood used for gamma counting and Hct is the hematocrit determined by the microcapillary method. The resulting constants were then multiplied by the 99mTc and 125I activity in each 100-μm-thick tissue slice to find the total volume of RBCs or plasma (i.e., $V_{\text{RBC}} = K_{99mTc} \times V_{\text{99mTc}}$ counts/min, and $V_{\text{plasma}} = K_{125I} \times V_{\text{125I}}$ counts/min). Tissue hematocrit in the slice was then calculated using these values (i.e., $\text{Hct} = V_{\text{RBC}} / (V_{\text{RBC}} + V_{\text{plasma}})$).

Aggregation I: Pluronic F-98 coating to increase RBC aggregation. In the RBC suspensions used for exchange in the aggregation I group, RBC aggregation was increased by employing a recently developed technique whereby poly(ethylene glycol)-poly(propylene glycol)-poly(ethylene glycol) copolymers (Pluronics) that possess the ability to self-associate above a specific temperature are covalently attached to the RBC surface. The details of the method are described elsewhere (6). Briefly, RBCs and plasma were separated from anticoagulated (EDTA, 1.5 mg/ml) donor guinea pig blood by centrifugation at 1,400 g for 6 min. RBCs were washed three times with

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isotonic PBS (pH 7.4), resuspended in 30 mM triethanolamine buffer (290 mosmol/kg, pH 8.60) at a hematocrit of ~0.1 l/l, and the suspension cooled to 4°C. The low value of hematocrit (i.e., ~0.1 l/l) was necessary to obtain an even coating of RBCs with Pluronic and to avoid the formation of cross-linked aggregates of coated RBCs. Immediately before the use of RBCs, a reactive succinimidyl carbonate derivative of Pluronic F-98 [13 kDa, 80% poly(ethylene glycol)] was dissolved in 4°C hypotonic phosphate buffer (50 mM NaH₂PO₄ and 60 mM NaCl, pH 5) at a concentration of 10 mg/ml and added to the RBC suspension to obtain a final concentration of 0.025 mg/ml. The suspension was incubated for 2 h at 4°C with continuous gentle mixing. Pluronic-coated RBC were then separated and washed three times with isotonic PBS by gentle centrifugation (400 g, 5 min) and then resuspended in native plasma at 0.4 l/l hematocrit.

All Pluronic-coating procedures were performed at 4°C, i.e., below the critical micellization temperature (CMT), at which a phase transition occurs from predominantly single, fully hydrated copolymer chains to micelle-like structures. At a temperature above the CMT, RBC aggregation occurs due to the formation of micelle-like structures between adjacent Pluronic-coated RBC, with the strength of aggregation dependent on both the Pluronic concentration at the RBC surface and on the physicochemical characteristics (e.g., molecular weight and CMT) of the selected Pluronic (1). Although Pluronic-coated RBC do not have an enhanced tendency to adhere to noncoated blood cells, they can form mixed aggregates, i.e., coated plus noncoated RBC, when lightly coated with Pluronic F-98 and suspended in an aggregating medium (1). Pluronic-F-98 RBCs do not exhibit an enhanced tendency to adhere to cultured vascular endothelial cells (unpublished observations).

Aggregation II: addition of dextran to increase RBC aggregation. RBC aggregation in the suspensions used for exchange in the aggregation II group was increased by suspending washed cells at a hematocrit of 0.40 l/l in plasma containing 1 g/dl of Dextran 500.

Microscopic and rheological studies. Evaluation of hemorheological parameters subsequent to the exchange transfusions was carried out by using blood samples obtained before the freezing of hearts. Wet-mount preparations of dilute RBC suspensions in plasma were examined and photographed by using bright-field light microscopy. Erythrocyte sedimentation rates (ESR) were measured by using Westergren sedimentation tubes, and RBC-plasma suspensions were adjusted to a hematocrit of 0.1 l/l. Plasma viscosity was measured at 1.500 s⁻¹ shear rate using a Wells-Brookfield cone-plate viscometer (Brookfield, Middleboro, MA). Whole blood viscosity was also measured by using the same device at shear rates between 37.5 and 1,500 s⁻¹. All viscosity measurements were performed at 37°C.

Statistics. Results are means (SD), except where stated otherwise (e.g., Fig. 5). Comparisons between two means were made by Student’s t-test, and the relationships between the depth of tissue and tissue hematocrit were tested by linear and nonlinear regression analysis.

RESULTS

Hemorheological alterations after exchange transfusions. In both aggregation I and II groups, RBC aggregation was found to be enhanced as determined by microscopic examination. In contrast to the control group where minimal aggregation was observed, RBCs in the aggregation groups formed aggregates with relatively few cells not associated with an aggregate; qualitative estimates via microscopy suggested essentially the same level of aggregation in the two groups. However, the appearance of the RBC aggregates differed somewhat between the two groups: those in the aggregation II group consisted of linear arrays of cells (i.e., “classic” rouleaux formation) with some secondary branching, whereas those in the aggregation I group tended to be slightly less regular with cells exhibiting linear and branched face-to-face aggregation (i.e., rouleaux) and some face-to-side structures. Such differences were anticipated due to the differing nature of the aggregation process between the two groups (1, 27). The aggregation strength and aggregate morphology of RBCs in the aggregation I group precluded measurements of aggregation with the use of an available photometric aggregometer (5), and hence ESR tests were employed. RBC sedimentation rates provided quantitative evidence for the enhanced aggregation (Fig. 1). Both aggregation groups had significant, approximately twofold, increases in aggregation versus control with no significant difference in ESR among these groups.

Whole blood viscosity values over the entire shear-rate range were higher in the aggregation II group than in the control and aggregation I groups (Fig. 2), reflecting the higher plasma viscosity in the aggregation II group (Fig. 3). In contrast, whole blood viscosity was only higher in the aggregation I group compared with the control group at the lowest shear rate. Although the lowest shear rate used in this study (i.e., 37.5 sec⁻¹) cannot be considered as representing low-shear blood viscosity (e.g., 1 s⁻¹ or less), the significantly enhanced blood viscosity at this shear rate, combined with the unaltered plasma viscosity (Fig. 3), does reflect a higher disaggregation shear rate for cells in the aggregation I group (4, 23).

Fig. 1. Erythrocyte sedimentation rate measured for blood samples obtained after exchange transfusions in 3 groups. Pluronic F-98-coated cells were used in aggregation I group, and 500 kDa dextran were used in aggregation II group. Values are means (SD). **P < 0.01, difference from control.

Fig. 2. Whole blood viscosities measured at shear rates between 37.5 and 1,500 s⁻¹ for blood samples obtained after exchange transfusions in 3 groups. Pluronic F-98-coated cells were used in aggregation I group, and 500 kDa dextran were used in aggregation II group. Values are means (SD); 3 curves are significantly different from each other by 2-way ANOVA. Variation between groups is F = 10.6; variation between shear rates, F = 24.4.
Myocardial hematocrit values. Systemic hematocrit values together with tissue hematocrit values in the subepicardial and subendocardial layers are presented in Table 1, and subepicardial and subendocardial myocardial tissue hematocrit values expressed as a percentage of systemic hematocrit are shown in Fig. 4. In both aggregation groups, the subepicardial hematocrit was statistically less than the systemic hematocrit ($P < 0.05$ or better), with the largest decrease (i.e., 17%) noted for the aggregation I group. The ratios of subepicardial to systemic hematocrit did not differ among groups (Fig. 4). The mean subepicardial hematocrit value for the aggregation II group was slightly higher than the corresponding values for the control and aggregation I groups (Table 1), but the differences were not statistically significant by one-way ANOVA. In all groups the subendocardial hematocrit was significantly less than the systemic hematocrit ($P < 0.05$), with a 22% decrease for the aggregation I group and a 26% decrease for the control and aggregation II groups. Neither the absolute subendocardial hematocrit values nor the ratio of subendocardial to systemic hematocrit differed among groups.

Although the absolute values of subepicardial and subendocardial hematocrit did not differ among groups, there were marked differences in the ratio of subendocardial to subepicardial hematocrit (Table 1): 1) Control group ratio was 0.82 ($P < 0.01$); 2) aggregation I group ratio was 0.94 ($P > 0.10$); and 3) aggregation II group ratio was 0.79 ($P < 0.01$). Thus the decrease in tissue hematocrit from epicardium to endocardium was significantly less than unity only in the control and aggregation II groups, whereas the small difference between the two hematocrits was not significant for the aggregation I group.

Figure 5 presents myocardial tissue hematocrit values for each group, expressed as a percentage of the subepicardial hematocrit (%$\text{Hct}_{\text{epi}}$), for various tissue slices between the subepicardial and subendocardial layers. The percentage was calculated for each animal and then averaged for the group to obtain the mean ± SE. In Fig. 5, the subepicardial layer corresponds to a normalized depth of 0 and the subendocardial layer closest to the endocardium corresponds to a normalized depth of 1. Inspection and analysis of these results lead to the following observations. First, two-way ANOVA analysis indicated a significant difference among the three curves. Second, the control group data demonstrate the anticipated existence of a tissue hematocrit gradient, with the hematocrit value in the slice closest to endocardium being significantly lower ($P < 0.01$) than that in the subepicardial layer. The gradient was not linear but appears to initially decrease rapidly and then reach a stable level at ~80% of %$\text{Hct}_{\text{epi}}$ at a normalized depth of ~0.5. Third, the aggregation I group data, i.e., RBCs coated with Pluronic F-98, appear to be essentially independent of position within the myocardium, with a slight tendency for decreased relative hematocrit in the two slices nearest the subendocardial layer. The average value of relative hematocrit for the 10 slices (99.1 ± 2.1, mean ± SE) did not differ from 100% (i.e., unchanged from subepicardial hematocrit). Fourth, the aggregation II group data demonstrated behavior intermediate between the control and aggregation I groups; i.e., over the first half of normalized depth, the relative hematocrit values were close to 100% and thus did not differ meaningfully from those for the aggregation I group. However, beyond a depth of ~0.5, the relative hematocrit decreased sharply and was coincident with the control group at the subendocardial position.

Table 1. Systemic Hct, subepicardial, and subendocardial tissue Hct values in control, aggregation I, and aggregation II groups

<table>
<thead>
<tr>
<th>Group</th>
<th>$n$</th>
<th>Systemic Hct, %</th>
<th>Subepicardial Hct, %</th>
<th>Subendocardial Hct, %</th>
<th>$\text{Hct}<em>{\text{endo}}/\text{Hct}</em>{\text{epi}}$, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7</td>
<td>0.38 (SD 0.03)</td>
<td>0.34 (SD 0.05)</td>
<td>0.28 (SD 0.05)†‡</td>
<td>81.7 (SD 0.09)</td>
</tr>
<tr>
<td>Aggregation I</td>
<td>6</td>
<td>0.40 (SD 0.03)</td>
<td>0.33 (SD 0.07)†</td>
<td>0.31 (SD 0.05)*</td>
<td>94.8 (SD 0.19)</td>
</tr>
<tr>
<td>Aggregation II</td>
<td>6</td>
<td>0.41 (SD 0.03)</td>
<td>0.38 (SD 0.05)*</td>
<td>0.30 (SD 0.10)‡</td>
<td>78.8 (SD 0.21)</td>
</tr>
</tbody>
</table>

Values are means (SD); $n$, number of hearts. $\text{Hct}_{\text{endo}}/\text{Hct}_{\text{epi}}$, subendocardial-to-subepicardial Hct ratio. *$P < 0.05$ and †$P < 0.01$, difference from systemic Hct, respectively; ‡$P < 0.01$, difference from subepicardial Hct in the same group.
DISCUSSION

The reversible aggregation of RBCs into multicell structures is a normal physiological process that occurs at low shear rates or at stasis and only in the presence of sufficiently large macromolecules in the suspending phase. Therefore, plasma composition is an important determinant of the degree of RBC aggregation (26). Additionally, it has been demonstrated that RBC surface properties play a significant role in RBC aggregation (27). Experimental and clinical data suggest that alterations of plasma composition, especially elevated levels of high-molecular-weight proteins (e.g., fibrinogen and macroglobulins), can lead to enhanced RBC aggregation (23, 27), and thus the circulatory effects of RBC aggregation are of clinical and physiological interest. The current study addressed the effects of enhanced RBC aggregation on myocardial hematocrit distribution, with the specific aim of examining possible differences between enhanced aggregation induced by two different experimental approaches. Recently developed methods for Pluronic coating of RBCs enabled the use of these two approaches (1).

In this study, the aggregating tendency of plasma was increased by the addition of Dextran 500, and thus the aggregation II group represents the use of altered plasma as the suspending media. Because the experimental protocol involved a 40% exchange transfusion of RBCs suspended in plasma containing 1 g/dl Dextran 500, this exchange should have resulted in a final plasma dextran concentration of ~0.4 g/dl. As anticipated, the addition of this high-molecular-weight macromolecule resulted in an increase of plasma viscosity (16%, Fig. 3) and markedly enhanced RBC aggregation (i.e., 101% increment in ESR, Fig. 1). Neither plasma viscosity nor plasma protein concentration was altered in the aggregation I group that received exchange transfusions with Pluronic F-98-coated RBC suspensions, yet RBC aggregation was enhanced to a similar degree (110%). Therefore, the use of these two methods for altering RBC aggregation represents a novel approach for comparing the effects on tissue hematocrit of enhanced aggregation induced by modified plasma composition or by modified RBC surface properties. The left ventricular myocardium is a useful tissue for such studies because it is possible to define tissue hematocrit values relative to the anatomic position of the tissue (2, 33).

The major finding of the current study is clearly shown in Fig. 5. The normal, control group hematocrit profile, with a significant decrease of hematocrit when traversing from subepicardial to subendocardial layers, is markedly altered by enhanced RBC aggregation. First, when aggregation is increased on using Pluronic F-98-coated RBC suspended in native plasma (aggregation I group), hematocrit differences across the myocardium are essentially abolished. Second, when RBC aggregation is increased on using a high-molecular-weight polymer (aggregation II group), hematocrit remains at subepicardial levels for ~60% of the myocardial thickness and then rapidly decreases to the control level in the subendocardial layer. Our results thus indicate that myocardial hematocrit profiles are sensitive to both RBC aggregation and changes of plasma viscosity associated with increased RBC aggregation. Tsai and coworkers (31, 32) have examined the microcirculatory effects of elevated plasma viscosity in extreme hemodilution produced by exchange transfusion with dextran solutions. Using a hamster skinfold model that allowed direct microscopic observation, they observed that functional capillary density was significantly higher when hemodilution was done using a high-viscosity Dextran 500 than when using a low-viscosity 70-kDa dextran. Our results (Fig. 5) partially support their observations: when using Dextran 500 and thus increasing plasma viscosity, hematocrit was maintained at subepicardial levels for ~60% of myocardial thickness, whereas the control group values were markedly lower over the same region. However, enhanced aggregation without increased plasma viscosity (aggregation I group) was more effective in maintaining hematocrit at subepicardial levels.

In the control group, the tissue hematocrit value at the subepicardial layer of the left ventricular wall was 92% of the systemic hematocrit (Fig. 4). This value is reasonably close to the average value previously reported for epicardial layers of rat left ventricular myocardium (2, 3, 33). A lower value (82%, Fig. 4) was found in the corresponding tissue samples obtained from the group with enhanced RBC aggregation but unaltered plasma composition (aggregation I). Based on previous observations of Fahraeus (12), Cokelet and Goldsmith (10), and others (11, 17, 21, 22), enhanced aggregation could result in lower tissue hematocrit values by promoting RBC axial migration and plasma skimming in smaller vessels. However, it has also been argued that the effects of RBC aggregation might be influenced by the relations between transit times of blood through the circulatory network and time constants of RBC aggregation (7, 8), thereby limiting the influence of aggregation on hemodynamic mechanisms favoring lower tissue hematocrit.
Although the main difference between the two methods to increase aggregation is assumed to be related to the plasma viscosity increase in the aggregation II group (Fig. 3), there does not appear to be experimental evidence suggesting that the alterations in plasma viscosity can affect the radial distribution of RBCs in vessels and hence tissue hematocrit. However, it can be speculated that increased plasma viscosity may lead to altered blood vessel diameters via its interference with endothelial cell vasomotor mechanisms that are dependent on wall shear forces (6, 13). Therefore, it would be interesting to test the effects of enhanced plasma viscosity without increasing RBC aggregation by introducing low-molecular-weight dextrans (e.g., 40 kDa) into the circulation. This approach was not included in the present study but should be considered in future experiments. Another important difference between the two approaches for increasing RBC aggregation is related to the strength of the aggregates. Pluronic F-98 coating of RBCs is known to produce irregular aggregates that resemble those found in various pathological conditions. The strength of aggregation and aggregate morphology are dose dependent and, when using high concentrations for cell coating, can result in aggregates that require significantly greater shear forces to cause disaggregation (1, 35). Although the in vivo behavior of Pluronic F-98-coated RBCs has not been studied in detail, it can be speculated that aggregates of these RBCs may persist in various portions of the circulatory system to a greater extent than those induced by 500 kDa dextran. Such behavior might be expected to enhance phase separation and related mechanisms that lead to lower microvascular hematocrit values. The essentially identical ESR values for the Pluronic F-98-coated and dextran groups (Fig. 1) only indicate that, at stasis, RBC aggregate sizes are similar and do not address the strength of the aggregates.

Unlike tissue hematocrit in the control group, tissue hematocrit in both aggregation groups remained at subepicardial levels for ~50 to 60% of the wall thickness and then either continued to remain at about this level (aggregation I) or decreased rapidly to the control level (aggregation II). The functional consequences of these changes are, at least, twofold. First, the increase of tissue hematocrit versus that in the control group (Fig. 5) should be beneficial, inasmuch as the oxygen-carrying capacity of the blood is increased. However, in the aggregation II group, this beneficial effect no longer exists for myocardial layers closest to the subendocardium. Subendocardial layers are prone to the highest extravascular pressures that tend to limit blood flow (20), making this region the most prone to ischemia. Second, blood viscosity increases in an exponential manner with increasing hematocrit, and thus the increase of hematocrit versus that in the control group should result in higher blood viscosity (23). In turn, it seems logical to assume that flow resistance in these layers is increased, thereby adversely affecting blood flow downstream in layers closer to the subendocardium. Thus, although it is possible to conjecture that the higher overall hematocrit in the aggregation I group are of physiological value, the actual flux of oxygen-carrying RBCs may be reduced due to decreased blood flow caused by higher blood viscosity.

The observations and conclusions herein are most likely limited to the specific experimental parameters employed (e.g., type of polymer coating and of dextran) and should be extended with caution to other vascular beds. However, the method used to determine myocardial tissue hematocrit values should be applicable in other studies. The radionuclide method for tissue hematocrit was originally developed by using rats as the experimental animal (33). However, preliminary attempts to utilize the Pluronic-coating technique with rat RBCs were not successful due to the tendency of these cells to undergo a discocyte-echinocyte shape change during in vitro processing. This shape transformation was observed with control (uncoated) and Pluronic-coated RBCs and is thus not related to the Pluronic-modification of the RBC surface. Conversely, guinea pig RBCs are more resistant to in vitro processing and preserve their normal discocytic geometry in the suspensions used for exchange transfusions (35). Unfortunately, there do not appear to be any data in the literature for tissue hematocrit in guinea pigs, thus precluding comparisons to prior studies.

It is interesting to note that although all RBC in the aggregation II group were exposed to plasma containing Dextran 500, only ~40% of the cells in the aggregation I group were coated with Pluronic F-98, and hence only these cells exhibited an intrinsic tendency for increased aggregation. Such a situation seems relevant to a model for altered aggregability of RBCs due to local disturbances of the microvascular environment (15). Under such pathophysiological conditions, circulating blood contains a mixture of modified RBC draining from damaged tissue and normal RBCs. Although it is possible that RBC subpopulations with different rheological properties preferentially aggregate with RBCs having similar properties (29) and although it is likely that Pluronic F-98-coated RBCs preferentially aggregate with each other, the Pluronic F-98-coating conditions used herein do not preclude the possibility of mixed RBC aggregates (1). Thus, although it is possible that the injected, uncoated 99mTc-labeled cells do not equally participate in aggregation in the aggregation I group, these cells would be affected by local microcirculatory flow dynamics and hence should reflect the spatial distribution of all RBCs.

The data obtained in this study represent the relaxed diastolic state of the myocardium, because only hearts arrested in diastole were used for the measurements. Although it was possible to detect that some hearts were arrested in systole, the radionuclide activity in the tissue slices obtained from these organs was very low, mostly as background activity, reflecting the low blood volume in the intramyocardial vessels (20). Therefore, it was not possible to use such hearts for determining tissue hematocrit. However, because perfusion of the left ventricular myocardium occurs mostly during diastole (20), information relevant only to the diastolic period should be of high physiological relevance. The dynamics of the rapid freezing process also suggest the physiological relevance of our results. Given that the rapid cooling of the heart within the rigid plastic chamber filled with liquid nitrogen starts at the same time on all external surfaces, Vicaut and Levy (33) have convincingly argued that the freezing process should not have a significant effect on hematocrit distribution. Most large blood vessels (arteries and veins) lie on or near the external cardiac surface, and these vessels are rapidly frozen or are at least cooled to near 0°C. Blood viscosity in these vessels thus increases very rapidly, thereby trapping blood in place before the total freezing of the tissue.

Finally, it should be noted that the exact mechanisms underlying the myocardial tissue hematocrit gradient are not clearly understood, although hemodynamic conditions through
the thickness of myocardium have been the subject of several detailed studies (14, 24, 30). The most important factor that changes with myocardial depth, especially in left ventricular myocardium, is the intramyocardial, extravascular pressure, i.e., extravascular compression, equivalent to the intraventricular pressure at the subendocardial layer and lowest at the subepicardial layer. These pressures also determine perfusion pressures and shear forces at various depths (20). Differences in hemodynamic conditions are strongly expected to influence RBC distribution and phase separation in the vascular system, although the complexity of hemodynamic conditions in the myocardium and the mechanisms related to RBC distribution currently make it inappropriate to offer extensive speculations regarding the influence of enhanced aggregation on myocardial hematocrit gradient (9). Further experimental and theoretical studies in this area are therefore warranted.

GRANTS
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