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Contributions of collision rate and collision efficiency to erythrocyte aggregation in postcapillary venules at low flow rates

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Kim S, Zhen J, Popel AS, Intaglietta M, Johnson PC. Contributions of collision rate and collision efficiency to erythrocyte aggregation in postcapillary venules at low flow rates. *Am J Physiol Heart Circ Physiol* 293: H1947–H1954, 2007. First published July 6, 2007; doi:10.1152/ajpheart.00764.2006.—Red blood cell aggregation at low flow rates increases venous vascular resistance, but the process of aggregate formation in these vessels is not well understood. We previously reported that aggregate formation in postcapillary venules of the rat spinotrapezius muscle mainly occurs in a middle region between 15 and 30 μm downstream from the entrance. In light of the findings in that study, the main purpose of this study was to test two hypotheses by measuring collision frequency along the length of the venules during low flow. We tested the hypothesis that aggregation rarely occurs in the initial 15- μm region of the venule because collision frequency is very low. We found that collision frequency was lower than in other regions, but collision efficiency (the ratio of aggregate formation to collisions) was almost nil in this region, most likely because of entrance effects and time required for aggregation. Radial migration of red blood cells and Dextran 500 had no effect on collision frequency. We also tested the hypothesis that aggregation was reduced in the distal venule region because of the low aggregability of remaining nonaggregated cells. Our findings support this hypothesis, since a simple model based on the ratio of aggregatable to nonaggregatable red blood cells predicts the time course of collision efficiency in this region. Collision efficiency averaged 18% overall but varied from 0 to 52% and was highest in the middle region. We conclude that while collision frequency influences red blood cell aggregate formation in postcapillary venules, collision efficiency is more important.

red blood cells; hemorheology; venous vascular resistance

AT LOW SHEAR RATES, red blood cells in the blood of athletic species, but not sedentary species, tend to form clusters or linear stacks (rouleaux) (20). This feature has been attributed to a bridging phenomenon (7, 14) or to an osmotic exclusion effect between adjacent cells by macromolecules (1, 19). Previous studies in this laboratory have shown that in the venular network, where shear rates are normally low, aggregation creates an inverse relation between flow rate and venous vascular resistance. The small venules contribute importantly to this relationship (10). This property may be important in maintaining a constant capillary hydrostatic pressure in skeletal muscle in athletic species where flow rate undergoes large changes (5). The aggregation also appears to influence the distribution of red blood cells in the capillary network by causing plasma skimming at bifurcations (12, 13) or by plug-

ging capillaries and causing flow stasis at high levels of aggregation (16, 17).

While these previous studies indicate that aggregation is functionally important, relatively little is known about the process of aggregate formation in vivo. In a recent study (11), we examined the aggregation process in postcapillary venules, which is the site in the venous network where it first appears. In that study, we showed that aggregation occurs in a localized region of the postcapillary venule, $\sim 15\text{--}30\ \mu\text{m}$ from the vessel entrance. No aggregates formed in the initial region $<15\ \mu\text{m}$ from the entrance, and few formed in the distal region $30\text{--}50\ \mu\text{m}$ from the entrance. We proposed that aggregates did not form in the initial region $0\text{--}15\ \mu\text{m}$ from the entrance because the velocity profile was not fully developed and axial migration of the red blood cells was insufficient to cause collisions between cells in adjacent flow streams from the feeding capillaries (the entrance effect; Ref. 8). We also proposed that in the distal region $>30\ \mu\text{m}$ from the entrance all the cells whose properties were favorable for aggregate formation (the older cells) had already formed aggregates and the remainder were younger cells that had a much lower aggregability (18).

In addition to collision frequency and intrinsic aggregability, red blood cell aggregate formation is likely to be related to a number of other factors including shear stress in the surrounding medium, relative velocities, surface area of contact, and angular motions (tumbling) of the colliding cells. The combined effect of all factors including collision frequency determines collision efficiency, which is the fraction of total collisions that result in formation of aggregates (9).

From the findings in our earlier study, it appears that measurement of the collision frequency of red blood cells in the initial region $0\text{--}15\ \mu\text{m}$ from the entrance would provide a test of the hypothesis that aggregates seldom form because most red blood cells from adjacent flow streams do not come into contact. Collision frequency measurement would also provide a test of the hypothesis that in the distal region $30\text{--}50\ \mu\text{m}$ from the entrance the remaining nonaggregated red blood cells do collide but have a low capacity to form aggregates. The main purpose of this study was to test these two hypotheses by measuring collision frequency along the length of the venules at low flow rates. In addition, we examined the possible effects of red blood cell axial migration and infusion of Dextran 500 on collision frequency and the differences in velocity of adjacent cells on collision efficiency.

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MATERIALS AND METHODS

Experimental setup and animal preparation. For the analyses in this study, we utilized video recordings obtained from five animals in our previous study of red blood cell aggregation in postcapillary venules of the rat spinotrapezius muscle (11) and a similar additional study using two animals. A detailed description of the experimental setup and animal preparation is available in that report and is briefly summarized here. Animal handling and care were provided in accordance with the procedures outlined in the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health, 1996). The study was approved by the local Animal Subjects Committee.

Male Wistar-Furth rats (172 ± 32 g body wt) were anesthetized with 50 mg/kg pentobarbital sodium given by intraperitoneal injection, and the spinotrapezius muscle was prepared for study. The animal was mounted on a temperature-controlled microscope stage, and a postcapillary venule with a clear image and contrast along the length of the vessel was chosen for study. We used an intravital microscope with a 100-W mercury lamp, a $\times 40$ water-immersion objective, and a long-working-distance condenser. The microscopic setup provided a field of $200 \times 200 \mu\text{m}$ and a spatial resolution of $\sim 0.4 \mu\text{m}$. We recorded the movements of the red blood cells with a high-speed video camera at frame rates up to 2,250 frames/s.

Experimental procedures. In this study we obtained data at reduced arterial pressure before and after infusion of Dextran 500 (200 mg/kg) to induce aggregability at the level seen in normal humans (12). To obtain distinct images of both individual cells and aggregates in the postcapillary venules, we reduced systemic hematocrit by exchanging blood (1–2 ml) from the experimental animal with the same amount of plasma obtained from a donor animal. We reduced arterial pressure to ~ 50 mmHg for a 1-min period by removing blood from the carotid artery into a heparinized syringe and reinfusing it at the end of the period. A total of seven animals was used in this study. Five venules were used before and five after dextran infusion.

Determination of erythrocyte and vessel wall location. Using digital video edit software (Adobe Premier 5.1), we converted the digital video clips created in the control unit of the high-speed video camera to image files (TIFF or BMP format). From the image files we obtained the x - y coordinate data for the center points of each red blood cell image with an image analysis software package (SigmaScan Pro 5). Depending on the cellular velocity and framing rate, every fifth to fiftieth frame was chosen for the determination of instantaneous position of each red blood cell passing through the postcapillary venule. The center position of the cell in x - y coordinates was manually determined in two independent trials and then averaged. The error associated with marking the center of red blood cell images on the image analysis software was $\pm 0.5 \mu\text{m}$, as reported in our earlier study (11).

We also determined the location of the vessel wall, using five separate measurements to reduce human measurement error. The inner diameter (ID) of individual venules was determined using the location of the vessel wall. Figure 1A shows the location of vessel walls and center line in one postcapillary venule ($14\text{-}\mu\text{m}$ ID). As shown in Fig. 1, the postcapillary venule was divided into initial (0–15 μm), middle (15–30 μm), and distal (30–50 μm) regions, which were determined along the length of the center line, for comparison of collision frequency, red blood cell migration, and aggregate formation. The initial region began at the point where vessel diameter became constant, within the range of measurement error ($\pm 0.5 \mu\text{m}$). The center line represented the locus of a point equidistant from the vessel walls.

Radial migration. We used red blood cell movements from frame to frame to determine radial migration. Red blood cell movements were then plotted relative to the center line and the axes were modified as shown in Fig. 1B, where the central x -axis represents the center line. Figure 1B also shows three examples of red blood cell pathways in the venule.

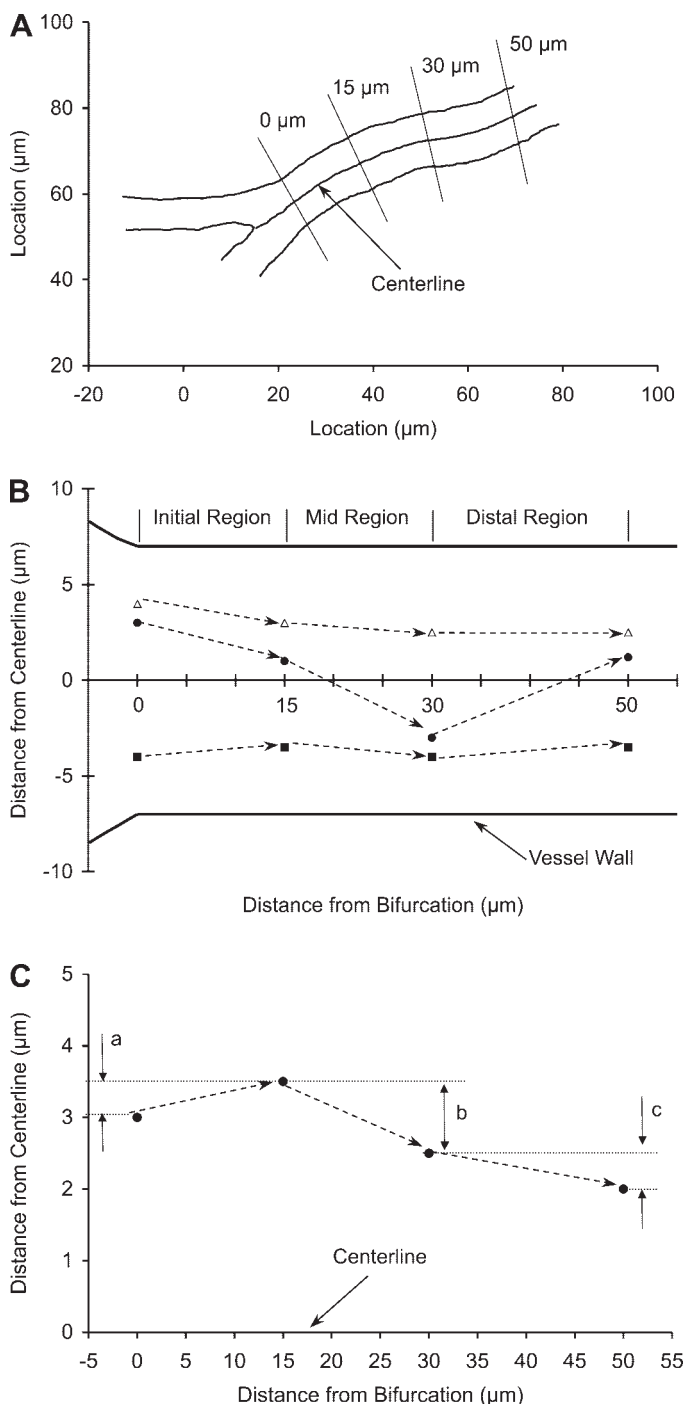


Fig. 1. Schematic diagram of venule, 2 feeding capillaries, and erythrocyte movements in venule. A: typical vessel wall geometry with center line added. B: replot of venule with center line as the x -axis. Examples of red blood cell pathways relative to center line in the initial, middle, and distal regions of the venule are shown. C: analysis of red blood cell movement for determination of radial migration: a , b , and c represent radial migrations of the red blood cell in each region of the venule. See text for details of analysis.

The radial migration of red blood cells in the three localized regions mentioned above was determined separately by taking into account radial movement of the cells in each region. For the example shown in Fig. 1C, the radial movements in each region are shown as a , b , and c , respectively. The radial migration of red blood cells in each region was then averaged individually. A positive value of

migration indicates red blood cell movement toward the center line of the venule.

Hemodynamics. Cellular velocity was determined from the location change of red blood cells in successive frames and the frame rate. The mean cellular velocity (V_{mean}) was calculated by averaging all the cellular velocities in the venule. The pseudoshear rate ($\gamma = V_{\text{mean}}/D$, in s^{-1} , where D is vessel diameter) was also determined before and after dextran infusion.

Criteria for erythrocyte collisions and aggregate formation. We considered a collision to have occurred between two red blood cells when the centers of the cells came within 4 μm of each other. For a single red blood cell and an aggregate, the criterion was distance between the center of the cell and the closest cell in the aggregate. When a cell separated from an aggregate, we counted each individual collision of that cell thereafter as a separate collision. As described in our previous study (11), we used the following criteria for formation of an aggregate: 1) the centers of two adjacent cells came within 4 μm , 2) the distance between centers of the two cells remained at ≤ 4 μm , and 3) the cells traveled in the same direction. Using these criteria, we calculated the number of aggregates formed in each 5- μm segment of the venule.

Determination of collision frequency and collision efficiency. We analyzed the collision frequency of 147 red blood cells in five venules before dextran infusion and 178 cells in five venules after infusion. We then tabulated the total number of collisions for each red blood cell based on the criteria described above and determined collision frequency of the red blood cells in each 5- μm segment of the venules taken together. From the data on aggregate formation and collision frequency, we then calculated the collision efficiency in each successive segment for all venules before and after dextran infusion.

Statistical analysis and data presentation. We utilized a paired t -test to determine differences in experimental and physiological parameters between normal and dextran-treated animals. A one-way ANOVA test was used to compare cellular velocities in three different segments. We tested for statistical difference between distributions with the two-sample Kolmogorov-Smirnov (K-S) test (available online at <http://home.ubalt.edu/ntsbarsh/business-stat/otherapplets/ks.htm>). A commercial software package (Prism 4.0, GraphPad) was used for all other statistical analyses. Data are reported as means \pm SD, and $P < 0.05$ was considered statistically significant.

The process of aggregate formation can be presented as a function either of time or of distance along the venule, as shown in our previous study (11). However, in the present study, data are presented as a function of distance to better understand the relation between the events studied and the vascular network itself. As noted above, collision and aggregation data were collated for all venules in each 5- μm segment.

RESULTS

Systemic values. Control arterial pressure was 113 ± 6.1 mmHg before dextran infusion and 109 ± 6.5 mmHg after infusion. The reduced arterial pressures were 53 ± 10.4 and 51 ± 11.7 mmHg in normal and dextran-treated rats, respectively, and the two values were not significantly different. As noted in MATERIALS AND METHODS, we reduced systemic hematocrit ($43 \pm 2.9\%$) by hemodilution with plasma. The reduced hematocrit before dextran infusion ($28 \pm 2.6\%$) was not significantly different from that after dextran treatment ($29 \pm 2.1\%$). Before dextran infusion the index (M) of aggregation, as measured with the Myrenne aggregometer, was 0.0, and it rose to 15.6 ± 4.2 after dextran infusion, which is similar to the level reported for the blood of healthy humans (15, 23).

Hemodynamics in capillaries and postcapillary venules. A total of seven postcapillary venules with a mean diameter of 11.5 ± 1.5 μm were used in this study. The tube hematocrit in

the venules before and after dextran infusion was $9.2 \pm 1.4\%$ and $9.6 \pm 2.9\%$, respectively; the two values were not significantly different. Each postcapillary venule was connected to two supply capillaries that had slightly different diameters. Mean velocity of red blood cells (204.7 ± 54.0 $\mu\text{m/s}$) in the larger capillary (7.9 ± 0.9 - μm ID) was significantly ($P < 0.01$) lower than that (258.3 ± 62.4 $\mu\text{m/s}$) in the smaller capillary (7.1 ± 1.0 - μm ID). There was no significant difference in V_{mean} in the postcapillary venules before (213.4 ± 58.7 $\mu\text{m/s}$) and after (182.9 ± 35.2 $\mu\text{m/s}$) dextran treatment. The pseudoshear rate γ was 20.9 ± 6.5 and 17.3 ± 6.7 s^{-1} before and after dextran infusion.

Process of aggregate formation. To examine the relation between experimentally determined collision efficiency and a theoretical model developed in this study, it was necessary to determine the time required for aggregate formation. We followed red blood cell movements in successive frames to determine how long the cells maintain a center-to-center distance of ≤ 4 μm after a collision. We found that if red blood cells satisfied the criteria for aggregation for ~ 35 ms after a collision, $\sim 95\%$ of the red blood cells continued thereafter in an aggregate form. Therefore, we considered 35 ms to represent the time required for aggregates to form. The first moment at which the cells collided was considered as the instant of aggregate formation. Approximately 50% of red blood cells formed aggregates in this study.

Spatial collision frequency in postcapillary venules. As described in the introduction, the main purpose of this study was to determine the effect of collision frequency on the spatial frequency of aggregate formation in localized regions of the postcapillary venules. Figure 2 shows the spatial frequency of collisions before and after Dextran 500 infusion for both red blood cells and aggregates as a function of distance from the entrance to the postcapillary venule. Before dextran infusion, the collision frequency was 26% in the initial 15- μm region, increased to 40% in the middle region (15–30 μm), and decreased moderately to 34% in the distal region. After infusion of dextran, the collision frequency was low (4%) in the first 5- μm segment and rose to 7% in the final 5 μm of the 15- μm initial region. Overall, this region accounted for only a small percentage (15%) of total collisions. The collision frequency increased greatly in the next two 5- μm sections to reach a maximum of 17%. Beyond that point (>25 μm) the collision frequency reached a plateau and then decreased modestly in the distal region. It is apparent that collision frequency is highly dependent on location in the venule.

Spatial distributions were quite similar before and after dextran infusion, although the collision frequency was slightly higher in the 10- to 15- μm region before dextran. Statistical comparison of the two patterns found no significant difference ($P = 0.14$) by the K-S test. Therefore, it seems that aggregate formation and the physical forces that lead to the formation did not influence the spatial frequency of collision. Since the aim of this study was to determine the effect of the spatial collision frequency on aggregate formation, subsequent analyses are limited to the condition after dextran infusion.

Temporal collision frequency in postcapillary venules. Since, as mentioned above, the process of aggregation is likely a time-dependent phenomenon, we examined the cellular velocity in each region and found significant differences ($P < 0.01$). As shown in Fig. 3A, the cellular velocity in the initial

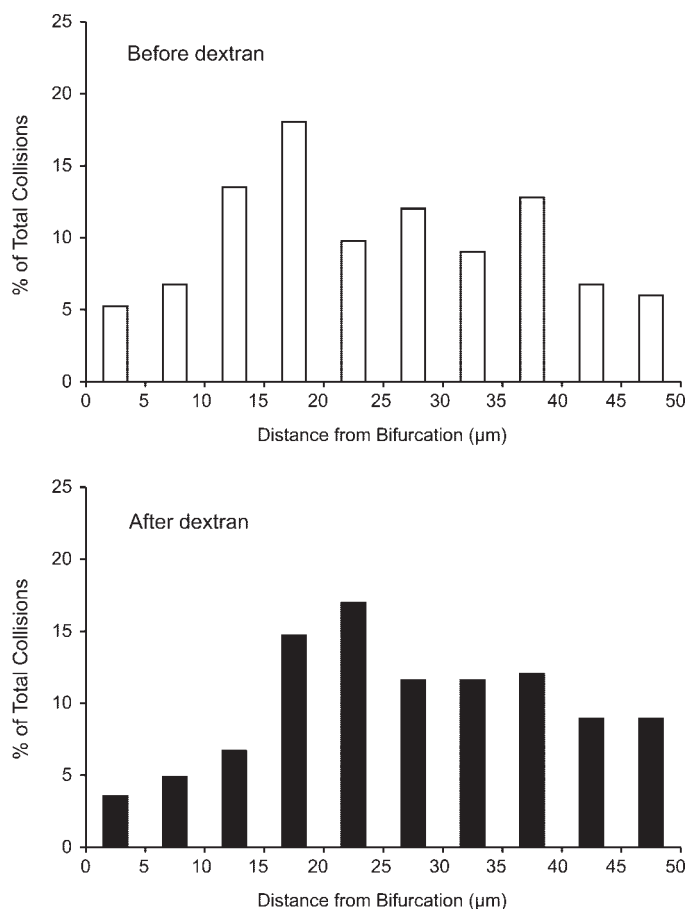


Fig. 2. Spatial distribution of total collisions in postcapillary venules. Note the similar gradual increase in the first 15–20 μm and gradual reduction in the distal 30- to 50-μm region before and after dextran infusion. There was no significant difference ($P = 0.14$) in the spatial distributions before and after dextran infusion.

15-μm region was highest and had largest standard deviation ($202 \pm 42.3 \mu\text{m/s}$). The velocity and standard deviation then decreased to 159 ± 27.2 and $149 \pm 23.8 \mu\text{m/s}$ in the middle and distal regions, respectively. In the initial region, the difference in red blood cell and plasma velocity may be greater than it is in the regions downstream. As considered in DISCUSSION, the variability in velocity may influence the probability of collision and aggregate formation.

Figure 3B shows the temporal variability of collisions in each region. The highest frequency (2.3/ms) took place in the middle 15-μm region, whereas the fewest collisions (1.0/ms) occurred in the initial 15-μm region. In the distal region, the collision frequency was 2.1/ms. Overall, the trend of temporal collision frequency is similar to that of spatial frequency.

Radial migration of erythrocytes. One factor that may influence the collision frequency is the radial migration of red blood cells, which would increase the probability that the cells from adjacent flow streams come into contact. Figure 4 shows the radial migration of red blood cells after induction of aggregation in the three regions of the postcapillary venules. Radial migration in the first 15-μm region ($1.0 \pm 0.69 \mu\text{m}$) was higher than in the other two regions (0.8 ± 1.55 and $0.1 \pm 1.06 \mu\text{m}$), but the collision frequency shown in Fig. 2B was not higher in this region, indicating that radial migration probably

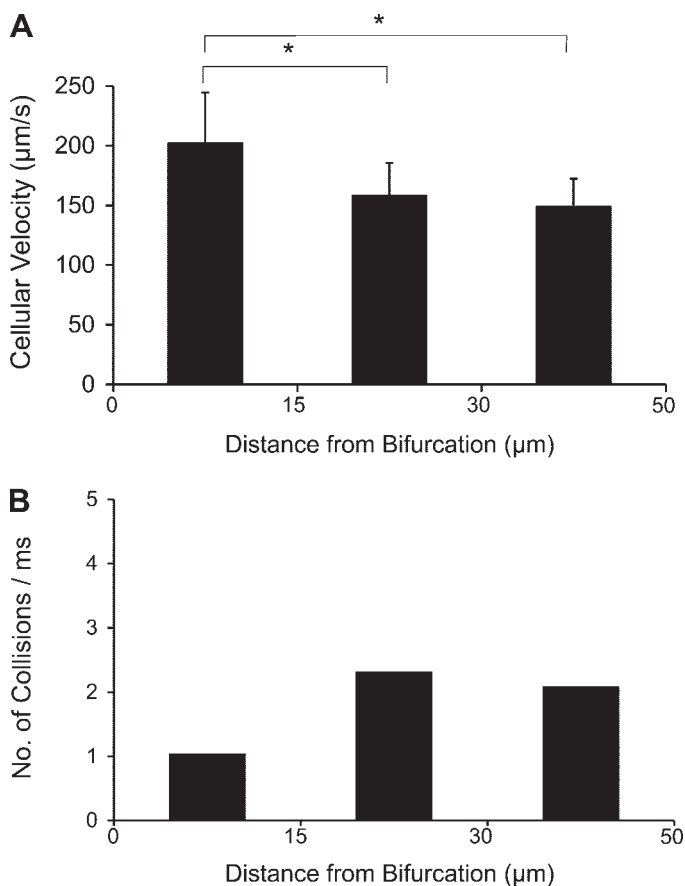


Fig. 3. Erythrocyte dynamics in postcapillary venules. A: cellular velocity in localized regions of postcapillary venules. Values are means \pm SD. $*P < 0.01$. B: collision frequency of erythrocytes in the localized regions.

did not contribute importantly to collision frequency. Radial migration in the middle 15- to 30-μm region was somewhat less than in the initial region, but collision frequency as seen in Fig. 2B was very high here. It is possible that radial migration may have contributed to collision frequency in this region. Radial migration was nil in the distal segment of the venule, and collision frequency was moderately less than in the middle

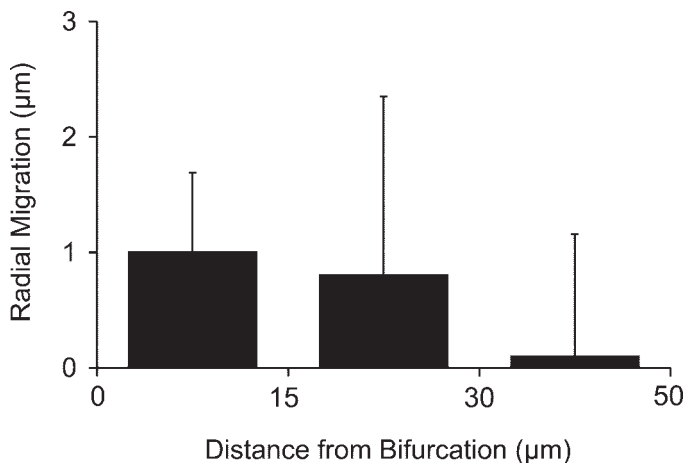


Fig. 4. Radial migration of erythrocytes in localized regions of postcapillary venules. Values are means \pm SD.

region. These findings indicate that radial migration had a consistent relation to collision frequency only in the middle and distal regions.

Orientation of erythrocytes in the venule. Collision frequency and aggregate formation in the three regions of the venule may be influenced by cell orientation. As shown in Fig. 5, there were two basic orientations. In the initial region, 76% of the red blood cells entered the venule in an alternating fashion from the two capillaries and traveled with no overlap relative to the flow direction (*orientation A*). The remaining 24% of the red blood cells entered concurrently from both capillaries with overlap and traveled with the flat surface approximately parallel to the direction of flow (*orientation B*). As shown in Fig. 5, *orientation A* increased to ~86% in the middle and distal regions because of increased separation in the axial direction in *orientation B*. For those cells traveling side by side in *orientation B* radial migration would increase the probability of collision, but it would not affect the great majority of the cells, which were in *orientation A*. This observation provides at least a partial explanation for the lack of effect of radial migration on collision frequency shown in Fig. 4. Therefore, it appears that red blood cell collisions occurred primarily by differences in velocity from cells arranged in *orientation A*.

Relation between collision frequency, aggregate formation, and collision efficiency. Figure 6 shows the relation between

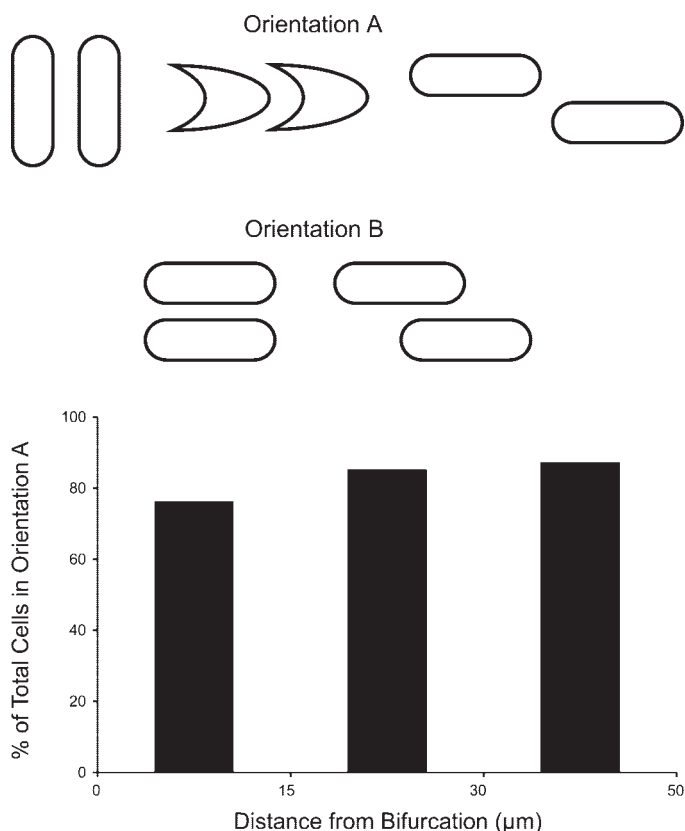


Fig. 5. Patterns of erythrocyte orientation in the flow stream. *Top*: schematic diagram of 2 types of red blood cell orientations. If red blood cells had any degree of overlap in the flow direction, we classified them as *orientation B*. Three examples of *orientation A* and 2 examples of *orientation B* are shown. *Bottom*: the fraction of red blood cells in *orientation A* increased as the cells traveled downstream.

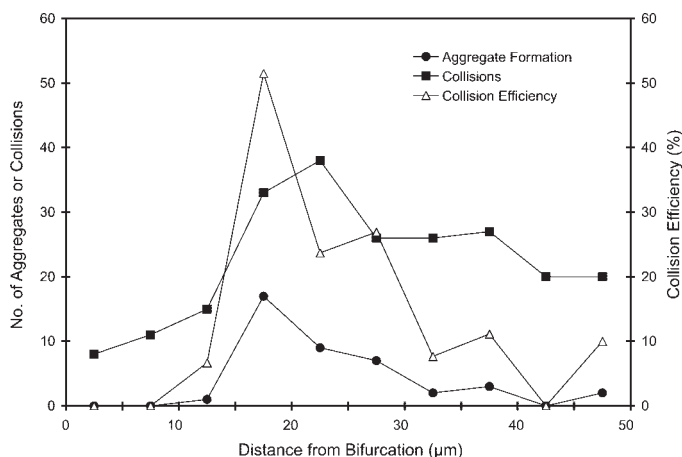


Fig. 6. Aggregation, collisions, and collision efficiency of erythrocytes in postcapillary venules. As previously noted, collision efficiency is defined as the fraction of collisions that produce aggregates. Similar trends of collision efficiency were observed in all venules.

collision frequency, aggregate formation, and collision efficiency (fraction of total collisions that result in formation of aggregates). As shown in Fig. 6, aggregate formation is confined almost completely to the middle region 15–30 μm from the entrance to the venule. Two red blood cells began to form an aggregate in the 10- to 15-μm segment of the initial region. Thus one aggregate (2% of aggregates) formed in the first 15 μm and 29% of aggregates formed in the distal 20-μm region. Comparing collision frequency and resultant aggregate formation, it is evident that increased collision frequency in the middle region is associated with an increased rate of aggregate formation. However, this factor alone does not explain the increased aggregate formation in this region. Collision efficiency is much greater in the middle region, reaching 52% in the first 5-μm section of this region. The combination of increased frequency of collisions and increased efficiency leads to a very steep rate of aggregate formation in the middle region.

DISCUSSION

Our previous studies in whole organs (6, 10, 21) have shown that venous vascular resistance in skeletal muscle increases as arterial pressure and flow rate decrease and that this phenomenon is due to red blood cell aggregation. Of particular relevance to the present study is the fact that this flow dependence of venous resistance has been shown to be quite significant in the small venules (10). Thus an examination of the aggregation process in this region is important to understanding the manner in which red blood cell aggregation exerts much of its effects on vascular resistance. We showed previously that in the larger venules velocity profiles of blood at low flow rates in rat spinotrapezius muscle became more blunted after dextran infusion (2) and this was associated with red blood cell aggregation (4). Also, we determined that red blood cells entering a venule from a smaller branch tend to travel near the wall of the larger vessel (3). Both of these phenomena would also have the effect of increasing the vascular resistance at low shear rates in the larger venules when aggregates are present. The present study provides new information on how hydrodynamic factors such as collision frequency, collision efficiency, and entrance

effects influence the process of aggregate formation in the postcapillary venule region.

Recently, we reported that aggregate formation in postcapillary venules mainly occurred in a middle region between 15 and 30 μm beyond a bifurcation point. In light of the findings in our earlier study (11), the primary goal of the present study was to determine collision frequency of red blood cells in postcapillary venules and its possible effect on aggregate formation at low pseudoshear rates (17–20 s^{-1}). We examined this and other factors that might influence aggregate formation and thus contribute to the increase in venous resistance at low flow rates. Collisions between red blood cells are a necessary but not sufficient condition for aggregate formation. To our knowledge, the effect of collision frequency on red blood cell aggregate formation has not been studied previously *in vivo*. The analysis involved collisions of 178 red blood cells after dextran infusion in a 50- μm region in five venules, or an average of about four collision events per 5- μm segment in each vessel. For the purpose of comparison, we have focused on frequency distributions in the three regions, the 15- μm initial, 15- μm middle, and 20- μm distal regions. Since the mean velocity of red blood cells in the postcapillary venules was ~ 0.2 mm/s in this study, if we assume that red blood cells with 6- μm diameter are uniformly distributed edge to edge the present analyses would provide information on red blood cell dynamics in a time window of ~ 5 s.

It should be noted that technical limitations may lead to errors in estimating the number of collisions and aggregates. The nature of our measurements did not enable us to determine whether cells meeting our criteria for aggregate formation, as described in *Criteria for erythrocyte collisions and aggregate formation*, are bonded by macromolecular bridging (7) or osmotic exclusion (17), the two proposed mechanisms for this phenomenon. In our earlier study (11) we found that 11% of red blood cells met our criteria for aggregation in the absence of Dextran 500 while 53% met this criteria in the presence of Dextran 500, which is known to produce aggregation (7). We conclude therefore that $\sim 80\%$ of the events that we classify as aggregation probably represent bridging or osmotic effects between adjacent red blood cells. We also note that close proximity of adjacent cells, whether or not bonding is involved, could have hydrodynamic consequences. That is, if two red blood cells are in close proximity and travel together, the hydrodynamic effects may be similar in the presence and absence of bonding.

We considered a collision between two cells to have occurred when the plasma gap became less than the spatial resolution, which in our study was ~ 0.4 μm . Another limitation was the absence of information on the z -coordinate, which might result in measurement error due to overlap of cell images. Both of these errors would tend to overestimate the number of collisions or aggregates. However, except as noted below, in this study it was the relative frequency among different segments of the venule and not the absolute number that was important. Therefore, we do not believe that such errors would compromise interpretation since we have no reason to expect that such errors would be systematically different among these segments.

In this study we tested two hypotheses, the first being that red blood cells rarely form aggregates in the initial 15- μm region of the postcapillary venule because of a lack of colli-

sions among the cells in that region. As shown in Fig. 2, only $\sim 15\%$ of the total number of collisions after dextran occurred in this region. Although we may have overestimated the absolute number of collisions in this region for the reasons cited above, it seems very unlikely that no collisions occurred. We found low spatial and temporal frequencies of collisions in this region relative to other regions after dextran, as shown in Figs. 2 and 3B. Thus our findings could be considered as partial support for the hypothesis that a low collision frequency reduces aggregate formation in the initial region. However, since only one aggregate formed, other factors may be more important.

As shown in Fig. 3A, the standard deviation was greater and the absolute value of variability in velocity (calculated from the mean velocity and coefficient of variation) was also greater in the initial region (42.4 $\mu\text{m/s}$) than in the middle and distal regions (26.9 and 23.8 $\mu\text{m/s}$, respectively). As reported in RESULTS, cellular velocities in the two feeding capillaries were significantly different. This observation may explain the higher variability of cellular velocity in the initial region where flow has not been fully developed. A recent experimental study has shown that collision frequency among solid spherical particles (1.8 mm in diameter) in a rectangular channel is proportional to $V_r^{0.547}$ where V_r is the mean relative velocity among particles (22). The volume fraction of particles in that study was $\sim 1.5\%$. Assuming that the standard deviation is an indicator of V_r , we would expect the temporal collision frequency to be greater in this region than in the middle and distal regions, which was not the case. However, in a restricted region such as the venule results obtained in macrosystems may not be applicable.

The likelihood of a collision between two red blood cells in the postcapillary venule depends on their relative velocities as noted above and on their positions as shown in Fig. 5. For cells in *orientation B* it requires radial migration that brings the cells into contact, while in *orientation A* it requires a difference in velocity in the direction of flow. The high radial migration in the initial region shown in Fig. 4 would favor collisions only for the cells in *orientation B*.

Collision frequency after dextran infusion reached the highest level in the middle region, especially in the 15- to 25- μm segment. This presumably involved mainly cells in *orientation A* that constituted 85% of the cells at this point. This is also the region with the highest level of aggregate formation and a high level of collision efficiency as shown in Fig. 6.

However, it does not appear that the physical (attractive) forces of aggregation caused the higher frequency of collisions in this region, since a similar increase was seen in the 15- to 20- μm segment before Dextran 500 was given, as shown in Fig. 2. It is notable that the middle region is just beyond the entrance region, where flow is not fully developed.

Collision frequency in the distal region 30–50 μm from the entrance was slightly lower than in the middle region. Axial migration was nil in this region, in contrast to both the initial and middle regions, but the effect on collision frequency should be slight since only 13% of the cells in this region were in *orientation B*. Cellular velocity and variability were similar to the middle region and significantly lower than in the initial region.

We next examine factors that may have influenced aggregate formation. If collision frequency were the only factor in the initial region, we would have expected that 15% of the aggre-

gates would form there. Since aggregates are first seen in the region close to the end of the entrance effect ($\sim 17 \mu\text{m}$) (8, 11), it seems a logical possibility that the entrance effect is responsible. In the entrance region where laminar flow is not fully developed, cell tumbling would be more common and would reduce the possibility of joining the flat surfaces of the cells. Red blood cells are usually transiently deformed in the entrance region as they enter into a larger vessel, which would retard aggregation. An additional factor that may retard aggregation in the initial region is the time required for the aggregation process itself. For example, as the aggregation force increases between adjacent cells, plasma must drain out of the gap between them (9). We estimate that ~ 35 ms is required for the aggregation process to reach completion after the collision occurs. This factor, together with tumbling and variability in velocity, may prevent aggregate formation in the initial region.

In the 15- to 20- μm region, the collision frequency is high, as is the rate of aggregate formation as shown in Fig. 6. The main reason for the increased rate of aggregate formation is the collision efficiency, which suddenly rises to 52% in this region, substantially higher than in any other 5- μm section. Overall, the collision efficiency in the venule was 18%. The reason for the very high collision efficiency in this section is not clear, but it may relate to the first appearance of fully developed flow where the flat surfaces of red blood cells are in a stable and parallel arrangement as shown in Fig. 5. In this region, cells would be traveling at similar velocities and a trailing cell that is traveling slightly faster than a leading cell would have a low impact force on contact with the leading cell, a circumstance that would favor aggregate formation. As aggregates form, the number of aggregatable cells remaining is depleted, leading to a drop in collision efficiency in the 20- to 30- μm segment.

The second hypothesis we tested is that aggregate formation in the region 30–50 μm from the origin is much less than in the middle section where most aggregates formed because the cells in the distal region have a much lower intrinsic aggregability. Meiselman and coworkers (18) have shown that aggregability does vary in a cell population, being threefold higher for older cells compared with younger cells. In our study, the older cells may have a greater tendency to aggregate in the presence of Dextran 500. Our data are consistent with the hypothesis that aggregability of the cells was the principal reason for low aggregate formation in the distal region. It is notable that axial migration was substantial in the central region but nonexistent in the distal region. The number of collisions was similar in both regions, although the collision frequency per unit length was lower in the distal region. From these data, as in the comparison for the initial and middle regions, it is difficult to find evidence that axial migration influenced collision frequency.

If the drop in collision efficiency in the 20- to 50- μm section is due entirely to a decrease in the fraction of aggregatable particles (single aggregatable cells plus aggregates), it should be possible to calculate the decline in collision efficiency downstream in the time domain, using the fraction in the 15- to 20- μm segment and the time required for aggregate formation. To determine collision efficiency, the probability that two particles will form an aggregate when they collide, we first assumed for the sake of simplicity that each collision between two aggregatable particles leads to formation of a single aggregated particle but that no aggregation results from colli-

sions with nonaggregatable particles. The likelihood of aggregate formation following a collision depends on the fraction of particles that are aggregatable. Thus the collision efficiency is the same as the probability that one aggregatable particle will collide with another aggregatable particle and can be determined from the following equation:

$$\text{collision efficiency} = \frac{m \cdot (m - 1)}{(m + n) \cdot (m + n - 1)} \quad (1)$$

where m and n are the numbers of aggregatable and nonaggregatable particles, respectively. The total number of red blood cells was 178, as mentioned in MATERIALS AND METHODS. Next, m and n were determined based on our assumption of the initial percentage of aggregatable cells. For example, when we assumed that 70% of red blood cells were aggregatable, which provided the best fit to the data shown in Fig. 7, m and n were 125 and 53, respectively. Since the time required for an aggregate to form was ~ 35 ms, we successively calculated the collision efficiency (Eq. 1) for each time interval (35 ms) as shown in Fig. 7. To compare the predicted values with experimental findings shown in Fig. 6, the spatial collision efficiency shown with each 5- μm segment was converted to the time domain, using the mean cellular velocity in the middle and distal regions. Since the number of separate aggregatable particles decreased as aggregates were formed while the number of nonaggregatable particles remained the same, the collision efficiency fell over time.

The predicted results in the time domain beginning with 50%, 60%, and 70% aggregatable red blood cells are shown in Fig. 7 together with the experimental findings in the time domain. With 50% aggregatable cells at the outset, the model predicts that 32% of total cells would form aggregates. Increasing the initial value to 60% raises that value to 45%, which is close to our experimental finding of 50% but predicts a relatively low efficiency (36%) initially compared with the experimental value of 52%. An initial value of 70% provides the closest overall fit to the experimental data, as it predicts a collision efficiency of 50% in the first region and predicts that

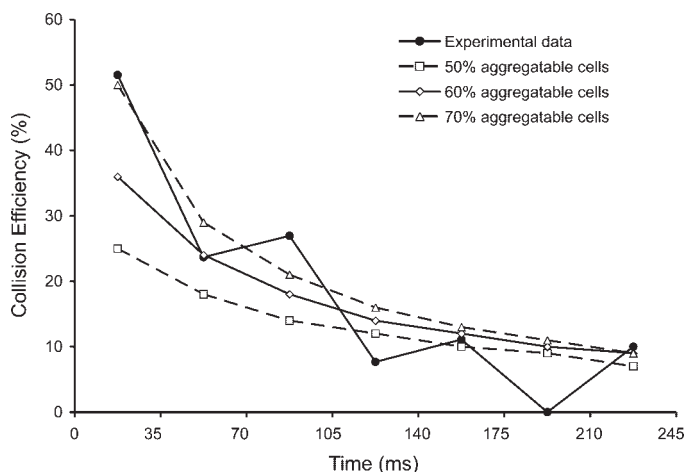


Fig. 7. Comparison of experimental data and collision efficiency determined from a probability calculation as described in the text. Experimental data were converted to the time domain for comparison with theoretical predictions. As shown, the initial percentage of aggregatable cells (50%, 60%, and 70%) led to differences in the time course of collision efficiency, with the 70% value providing the closest fit.

58% of total cells would form aggregates. While it is highly likely that aggregability is a monotonic rather than a step function of cell age and that aggregatable cells do not always form aggregates on colliding, the overall fit of the prediction with an initial value of 70% aggregatable cells indicates that these assumptions and the 35-ms aggregation time are reasonable first approximations. The time window of 35 ms was determined based on data obtained from 178 red blood cells after dextran infusion. If two red blood cells stayed together with $<4\text{-}\mu\text{m}$ center-to-center distance for a minimum of 35 ms, $\sim 95\%$ of those red blood cells satisfied our aggregation criteria afterward, as mentioned in RESULTS. Thus, with a longer time window (>35 ms), we would not expect to obtain a significantly different collision efficiency.

In conclusion, we have found that collision frequency and collision efficiency vary in different regions of the postcapillary venule, with efficiency being more important than frequency in determining aggregate formation. Collision efficiency appears to depend on entrance effects in the venule, the time required for aggregate formation, and the ratio of aggregatable to nonaggregatable cells. As blood flows through the middle and distal regions of the venule and aggregates form, collision efficiency decreases because of depletion of aggregatable cells. Efficiency also depends on the probability of a collision between two aggregatable cells and decreases disproportionately as the ratio of such cells to nonaggregatable cells in the bloodstream falls. This study also revealed that red blood cell collisions are due principally to differences in velocity of cells in the same flow stream rather than to radial movement of the cells.

The remaining question is how the aggregation process described above might increase vascular resistance in small venules. Two aspects may be considered. First, the process of aggregate formation itself could involve expenditure of energy. Second, the increase in particle size due to aggregation, particularly in these venules where the particle is a substantial fraction of the vessel radius, would create a more blunted velocity profile and increase energy loss. While energy would seem to be required for these processes, the magnitude and importance would require further study.

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