Velocity Profiles of Blood Platelets and Red Blood Cells Flowing in Arterioles of the Rabbit Mesentery


Velocity profiles were determined in rabbit mesenteric arterioles (diameter 17–32 µm). A good spatial resolution was obtained by using the blood platelets as small and natural markers of flow, providing for the first time in vivo detailed, quantitative information about the shape of the velocity profiles in microvessels. In some experiments red blood cell velocity profiles were recorded as well. Easy detection of the cells of interest could be achieved by labelling them selectively with a fluorescent dye and visualizing them by intravital fluorescence video microscopy, using flashed illumination. Pairs of flashes were given with a short, preset time interval between both flashes, yielding in one TV picture two images of the same cell displaced over a certain distance for the given time interval. Velocity and mean radial position of cells, flowing within an optical section around the median plane of the vessel, were determined. The shape of the velocity profiles of platelets and red blood cells was similar. The profiles were flattened as compared to a parabola, both in systole and diastole. Vessel diameter did not change measurably during the cardiac cycle. As an index of the degree of blunting of the profiles, the ratio of the maximal and mean velocity of the profile was used, which is 2 for a parabola and 1 for complete plug flow. The index ranged from 1.39 to 1.54 (median 1.50), and increased with vessel diameter. Calculations showed that the blunting of the profiles cannot be explained by an influence of the finite depth of the optical section. (Circulation Research 1986;59:505-514)

Detailed knowledge of the velocity profile in microvessels, i.e., the velocity distribution over the cross-sectional area of these vessels, is important for several reasons. First, adequate description of blood flow through small vessels requires information about the velocity gradients or shear rates in the fluid. Second, transport of cellular components in the blood is determined by both their distribution over the cross-sectional area of the vessel and the velocity profile. For instance, knowledge of the distribution of blood platelets in microvessels and their velocity profile allows the calculation of the rate of platelet delivery in hemostatic plug or thrombus formation in these vessels. Third, to estimate volume flow in microvessels photometric methods are widely used, employing an empirical factor derived from a model in which a parabolic velocity profile is assumed. However, in this approach an error will be made if in vivo the velocity profiles are more flattened.

In vitro studies in glass tubes on the velocity profiles of ghost cell suspensions or blood have yielded conflicting results: A parabolic profile, a flat profile at extremely low velocities but almost parabolic at higher velocities or blunt profiles. In addition, such studies have been performed only in tubes with a diameter larger than 30 µm.

Until now, precise measurement of velocity profiles in small blood vessels has not been performed in vivo for technical reasons. Photometric methods cannot be used to determine a profile because the system does not provide a direct measure of the red blood cell velocity in the plane of sharp focus. With high-speed cinematography, displacement of red blood cells can be followed during a short period of time. These types of measurements, however, are difficult because of the opacity and the large number of red blood cells present in the vessel. So far these measurements have yielded only a ratio between red blood cell velocities near the wall and the center of the vessel, or a qualitative impression of the shape of the velocity profiles because of the limited resolution. In a few studies laser Doppler velocimetry has been used to determine velocity profiles in microvessels but only in vessels with a diameter larger than 65 µm because of the relatively large sample volume in these systems.

In the present study velocity profiles were determined in arterioles with a diameter ranging from 17 to 32 µm, using blood platelets as a natural marker of flow. Recently, we developed a technique to label the circulating blood platelets in vivo with a fluorescent dye. Intravital fluorescence microscopy then allows easy detection of the individual blood platelets flowing in a microvessel amidst the excess of unlabelled red blood cells. Since platelets are small, disk shape cells that tend to align with flow, a good spatial resolution can be obtained to accurately assess the velocity profiles, allowing quantitative analysis over the full width of the profile except for a small zone (0.5 µm) close to the wall. A corollary the velocities of the few white blood cells observed during these measurements were assessed as well. In addition, in some experiments red
blood cells, labelled in vitro with a fluorescent dye, were injected to compare platelet and red blood cell velocities.

Materials and Methods

Experimental Set-Up

Seven rabbits of various breeds and either sex, ranging in weight from 3.5 to 4.0 kg, were anesthetized with intramuscular injections of ketaminehydrochloride (Vetalar, Parke Davis, 20 mg/kg body wt.) and 5,6-dihydro-2-(2,6-xylylidino)4H-1,3-thiazine-hydrochloride (Rompun 2% solution, Bayer, 8 mg/kg body wt.). Surgery was started 5–10 minutes after the second injection of these compounds, which was given 15–20 minutes after the first one. Anesthesia was maintained by injecting the same doses of the drugs every 30 minutes. Shortly following induction of anesthesia blood from a central ear artery was collected in disodium ethylene diaminetetraacetic acid (EDTA, 0.1 vol, 0.027 M), and the hematocrit and electronic platelet count were measured in duplicate as previously described.

The anesthetized animal was placed on an electrically heated (35° C) microscope table. Copper electrodes were inserted into the paws to record an electrocardiogram (ECG; Philips PM-system). The ECG was used to determine velocity profiles at a given moment in the cardiac cycle (see below). Through a small midline incision a short segment of distal ileum was brought outside the abdomen. The mesentery was spread over a siliconized glass plate, continuously superfused with a buffered Tyrode solution and kept at 37° C. The gut was kept moist with overlying wet gauze. Care was taken not to touch or stretch the mesentery itself. We only used preparations without microbleedings or capillary stasis and with minimal adherence of leukocytes to the wall of venules. In none of the preparations was spontaneous rhythmic changes in vessel diameter, was observed.

All observations were made with a Leitz intravital microscope adapted to telescopical imaging. In 9 arterioles (diameter 17–32 μm; Table 1) sites without upstream branch points within at least 6 vessel diameters were selected. At this distance from an upstream side branch, a disturbance of the velocity profile due to the bifurcation, if any, will be small. In the microcirculation the Reynolds numbers are far less than unity. Therefore, inertia forces are negligible and the entrance length will be about one vessel diameter. Curves immediately upstream or downstream of the site of measurement could not always be avoided, since in the unstretched mesentery most arterioles meander with curves in opposite directions shortly following each other. Each vessel site was examined with position of sharp focus in the median plane of the vessel, i.e., the focal position yielding the widest vessel diameter.

To be able to determine velocity profiles in both systole and diastole, instantaneous red blood cell velocity was assessed photometrically in 3 arterioles or to the determination of the velocity profiles, using a prism grating system. The time interval between the R-wave of the ECG and the moment at which the red blood cell velocity in the microvessel reached its maximum value (systolic phase) was approximately 190 msec in each of the three vessels. The diastolic phase coincided with the R-wave of the ECG.

Fluorescent Labelling and Fluorescence Microscopy

Fluorescent labelling of the circulating blood platelets has been described in detail elsewhere. In short, 2.5 ml of a solution (18.2 mM) of the fluorochrome acridine red (Chroma) was injected (lasting 20–30 seconds) through a polyethylene catheter placed in a marginal ear vein. With this procedure nearly all platelets are labelled without activation of the platelets by the dye or induction of gross ultrastructural changes. In addition, their capability to adhere and aggregate is maintained. During 2–8 minutes following the injection a clear microscopic image of individual platelets can be obtained by fluorescence microscopy, using illumination with flashes of short duration (< 0.1 msec) to prevent a smeared image due to moving of the platelets (see Figure 1A). The vessel wall and white blood cells are also labelled by the dye (see Figure 1B), but red blood cells are not. Generally white blood cells and platelets can be easily distinguished because of the difference in size and shape. The labelling of the vessel wall permits orientation within the microvasculature. In 3 animals the blood collected from the central ear artery was used to prepare fluorescently labelled red blood cells for injection into another animal. The blood (about 4 ml) was centrifuged at 5000 rpm for 10 minutes, the supernatant (including the buffy coat) removed and the red blood cells washed in physiological saline. In a concentration of about 15% (v/v) the cells were added to a solution of 100 mg fluoresceinisothiocyanate (FITC; Merck) in 150 ml phosphate buffer solution adjusted to a pH of about 8.2. The cells were incubated at room temperature for 2 hours when used at the same day, or overnight at 4° C when used the following morning. After incubation the cells were centrifuged and washed in excess physiological saline containing 1% bovine serum albumin (Serva). Prior to their injection into the lateral ear vein of the animal, the packed cells were diluted 1:1 in physiological saline.

Injection of the FITC-labelled red blood cells and determination of their velocities (see below), was always performed several minutes prior to the injection of the dye acridine red, which was used to label the blood platelets. The peaks of the absorption and emission spectra of acridine red are about 525 and 625 nm, respectively and of FITC about 490 and 525 nm, respectively. To obtain optimal results different filter sets have to be used (see below). The overlap between the two absorption spectra is such that, when using the FITC-filter set, acridine red present in the blood plasma was still able to cause sufficient background intensity to hamper the detection of the labelled red blood cells, which had a relatively weak fluorescence. A
Fluorescent platelets (A), a leukocyte (B) and red blood cells (C) flowing in arterioles, as observed with dual-flash illumination, yielding in one TV picture two images of the same cell, displaced over a certain distance during the time interval between the two flashes. The first flash was given in the blanking period of the TV camera. Arrowhead in A indicates the moment of the second flash (4 msec after the first one). Arrows indicate the direction of flow. In A and B only fluorescence microscopy was used. In C, fluorescence microscopy was combined with weak (transillumination) bright field microscopy to observe the vessel wall, which in this case was not labelled with a fluorescent dye. Level of sharp focus is in the median plane of the vessel. Note in C the triangular shape of the red blood cell at the left.

Determination of Platelet Velocity

To measure platelet velocity, pairs of flashes were given with a short, preset time interval between both flashes, yielding in one picture two images of the same platelet displaced over a certain distance for the given time interval (see Figure 1A). The first flash was given in the blanking period of the TV camera. In each experiment the time interval between the first and second flash (range 1–5 msec) was selected in such a way that the two concomitant images of platelets showed no or little overlap. The smallest displacement of the two platelet images, as measured (see below) near the vessel wall in the different experiments, ranged from about 2–4 μm. Within a measuring period (30–60 seconds) all flash pairs were given at a selected moment in the cardiac cycle (heart rates 130–200/min), using a delay unit (Tektronix TM) triggered by the R-wave of the ECG. The period of time between successive flash pairs (300–450 msec) was sufficiently long to ensure that each TV picture contained no information from the previous flash-pair and to render the chance of measuring a platelet twice negligible.

In the three arterioles, in which the time intervals between the R-wave of the ECG and the different phases of the velocity waveform in the microvessel were determined, platelet velocity profiles were assessed in both the diastolic (delay 0 msec) and systolic phase (delay 190 msec). During diastolic as well as systolic sampling the time interval between the flashes of a flash pair was the same. In the other arterioles a velocity profile was obtained only during the diastolic phase.

Off-line analysis was performed frame by frame. A transparency was placed over the monitor screen. Then the vessel walls, defined as left or right according to the direction of flow, were drawn. The length of the vessel segments analyzed, ranged from about 35–45 μm. To determine the velocity profile, the centers of gravity of the platelet images were identified by eye and marked by a point on a second transparency taped over the first one. These points were used to measure with a vernier calipers (scale division 0.1 mm, which was less than one fifth of the interline spacing on the monitor) the displacement of the platelet in the preset time interval and its relative radial position, defined as the mean of the relative radial positions of the two
images. Radial displacement of the platelet within the small time interval was on the average less than 2% of the vessel diameter. Although platelets can move in apparent contact with the wall, their center of gravity cannot come closer to the wall than about 0.5 \( \mu m \). Consequently, no data points could be obtained from this region. Velocities were displayed as a function of relative radial position, rendering the velocity profile.

Only images, that were reasonably sharp and clearly belonged to the same platelet, were used. This means that, with the high numerical aperture of the objective lens and the total optical magnification used, the data was obtained from an optical section around the median plane of the vessel with a depth of about 5 \( \mu m \) or less. Because of rotational symmetry, data obtained from the median plane of a vessel or, as an approximation, from a shallow section around the median plane, will be representative of the whole cross-sectional area.

**Determination of Leukocyte and Red Blood Cell Velocity**

During determination of the platelet velocity profiles once in a while a leukocyte was observed (see Figure 1B). The velocities and relative radial positions of the few white blood cells observed during each measuring period were assessed in the same way as for the platelets.

Velocity profiles of red blood cells were assessed in three arterioles, in two cases during the diastolic phase, in the other arteriole during the systolic phase. In addition, a platelet velocity profile was assessed in these arterioles during the same phase of the cardiac cycle. Determination of red blood cell velocities was similar to that of blood platelets, using the same range of intervals between both flashes. To observe the vessel walls, which in this case were not labelled with a fluorescent dye, weak (transillumination) bright field microscopy was combined with fluorescence microscopy (see Figure 1C). In addition, when drawing the vessel walls on a transparency for the analysis, the precise location of the wall was checked by comparison with pictures obtained by normal bright field microscopy before and after each measuring period and with pictures obtained by fluorescence microscopy during the subsequent determination of platelet velocity. The measuring periods ranged from 60–85 seconds. The smallest displacement of two red blood cell images, as measured near the vessel wall in the different experiments, ranged from about 5–7 \( \mu m \). Radial displacement of the red blood cells within the small time interval between both flashes was on the average less than 3.5% of the vessel diameter. Like platelets, red blood cells can be observed in apparent contact with the vessel wall. However, their center of gravity cannot come closer to the wall than about 2.5 \( \mu m \) and, hence, no data points could be obtained from this region. The depth of the optical section for the labelled red blood cells was assessed by focusing up and down through some red blood cells caught in small vessel segments where flow had stopped. The depth of the optical section for the FITC-labelled red blood cells was approximately 10 \( \mu m \).

**Curve Fitting and Statistical Analysis**

At present different theories exist to describe the flow pattern of blood in small tubes. All current theories are semiempirical and so far no theory has been proposed that is derived from the basic properties of blood cells and plasma. In order to describe in an objective way, the shape of the experimental profiles and a deviation from a parabola, if any, the following simple equation was used:

\[
V(r) = V_{\text{max}} (1 - |r/R|^b), \quad 1 \leq r/R \leq 1, \quad (1)
\]

where \( V(r) \) is the velocity at radial position \( r \), the vertical stripes denote absolute value, \( V_{\text{max}} \) is the velocity in the center of the vessel, and \( R \) is the radius of the vessel. The equation satisfies the no-slip condition at the wall. If \( K = 2 \), a parabolic velocity distribution is obtained. An increase in \( K \) yields a progressively flatter profile. The ratio of \( V_{\text{max}} \) and the mean velocity of the theoretical profile, \( V_{\text{mean}} \), is equal to \( (K + 2)/K \). This ratio can be used as an index of the degree of blunting of a profile, being 2 in the case of a parabolic profile and 1 in the case of complete plug flow, i.e., all layers of fluid are travelling at the same speed.

As mentioned before, no data points could be obtained from a small region close to the wall. The aim of the fit was not to predict the velocity distribution within the region close to the wall, where data points were absent, but to draw, as a form of objective eyeballing, a proper line through the experimental points. In order to improve the resemblance between the fitted line and the actual data points, the fits were no longer forced through zero velocity at the wall. In addition, asymmetry of the profiles, if any, was taken into account. To this end equation (1) was modified as follows:

\[
V(r) = V_{\text{max}} (1 - |a(r/R) + b| K), \quad a > 0, \quad (2)
\]

where \( a \) is a scale factor, allowing a non-zero intercept of the fit with the vessel wall, and \( b \) is a parameter correcting for a shift of the top of the profile, if any, away from the vessel center. If the scale factor \( a \) is smaller than 1, the intercept of the fit with the vessel wall will be positive and will increase towards the value of \( V_{\text{max}} \) when a approaches zero.

Estimation of the ratio of \( V_{\text{max}} \) and \( V_{\text{mean}} \) in the vessel using the profile described by Equation 2, will yield a value that is too low, because at the vessel wall fluid velocity will actually be zero. However, in the case of a good fit with the experimental platelet velocity profiles, the error will be limited since platelet velocities could be measured as close to the wall as 0.5 \( \mu m \). Simple calculation, using the data from Table 1 and assuming a linear decline to zero velocity at the wall within the region of 0.5 \( \mu m \) from the wall, showed that even in the worst case the error was less than 4%. Since parameter \( b \) was small (see "Results"), the ratio of \( V_{\text{max}} \) and \( V_{\text{mean}} \) was approximated by:

\[
V_{\text{max}}/V_{\text{mean}} = (K + 2)/(K + 2 - 2a^2). \quad (3)
\]
Fitting the data with a parabola and with Equation 1 or 2, was performed by linear and nonlinear regression, respectively, using modules 1R and 3R in the statistical package BMDP.22

To evaluate whether the velocity in the arterioles remained stationary during assessment of the profiles, a serial number was assigned to each measurement and the velocities of platelets or red blood cells flowing at a relative radial position of less than 0.3 were plotted against their serial number. Within the range of K and a values obtained, the differences in velocity between Vmax and the mean velocity of the theoretical profile, used an index of the degree of blunting of the profile, Equation 3.

Results

In the 7 animals the hematocrits ranged from 32 to 42% (mean 38%), and the electronic platelet counts from 215 to 594 x 10^7/liter (mean 352 x 10^7/liter), which are values within the normal range for rabbits.23-24

Platelet Velocity Profiles

In Figure 2 an example of a diastolic and systolic velocity profile is shown. Each dot represents the velocity of one platelet. An open square indicates the velocity of a leukocyte. No differences in vessel diameter could be detected between the diastolic and systolic phase. The same was found in the other experiments in which a systolic and diastolic velocity profile were determined.

Figure 3A shows another profile as well as the best fits with a parabola (K = 2) and with Equation 1, yielding a value of K = 4.02. The residuals of the fits are shown in Figures 3B and C, respectively. It is clear from the Figures 3A and B that the fit with a parabola is not good. The parabola underestimates the platelet velocities near the wall and overestimates the velocities in the center of the vessel. The same pattern of residuals:

- See for Vmax, i.e., the maximal velocity of the fitted curve, K and a Equation 2; for Vmax/Vmean, i.e., the ratio of Vmax and the mean velocity of the theoretical profile, used an index of the degree of blunting of the profile, Equation 3.

![Figure 2](image-url)
As is shown in the Figures 3A and C, the description of the data with Equation 1 was better than the description with a parabola. However, as indicated by the pattern of residuals presented in Figure 3C, the fit tended to underestimate the velocities in the center of the vessel and near the wall, and to overestimate the velocities at a relative radial position of about 0.5–0.8.

The values of the parameter K, as found by fitting with Equation 1, ranged from 3.8 to 5.9 in the different experiments with a median value of 4.55.

In contrast to the fits with a parabola and with Equation 1, a good description of the profiles could be obtained with Equation 2. An example is presented in Figure 4A, which shows another profile as well as the best fits with Equation 1, K = 5.9 and a = 1, and Equation 2, K = 2.4 and a = 0.83. The residuals of the fits are shown in Figures 4B and C, respectively. It is clear from the distribution of the residuals, that the fit with Equation 2 was better than that with Equation 1. A similar pattern, as shown in Figure 4C, was obtained in the other experiments. At each relative radial position within a vessel the residuals scattered around zero, with no systematic over or underestimation of velocities.

The values of V_{\text{max}}, K, and a, as obtained in the different experiments by a fit with Equation 2, are presented in Table 1. The absolute values of b as obtained in the different experiments ranged from 0.0003 to 0.038 (median 0.017). In addition, the values of the 

\[ V_{\text{max}}/V_{\text{mean}} \]

as calculated with Equation 3 are presented in Table 1. The values of the ratio of V_{\text{max}} and V_{\text{mean}} in both systole and diastole, were between 1.39 and 1.54, indicating that the profiles differ significantly from a parabola, in which case a value of 2 should have been found. The difference is caused by a higher velocity close to the wall than expected in the case of a parabola, which is indicated by parameter a, as well as by a flattening of the profile in the center of the vessel, which is indicated by parameter K. The small values of parameter b indicate that in the different experiments the top of the profile was positioned close to the center of the vessel.

With an increase in vessel diameter the value of V_{\text{max}}/V_{\text{mean}} used as an index of the degree of blunting of a profile, tended to increase, i.e., a less blunted profile, as can be seen in Table 1. A least squares linear regression of V_{\text{max}}/V_{\text{mean}} on vessel diameter, using the values obtained in the diastolic phase, yielded V_{\text{max}}/V_{\text{mean}} = 0.0090 \times \text{diameter} + 1.24 with a correlation coefficient of 0.71 (p < 0.05), suggesting a relation between vessel diameter and the degree of blunting of the velocity profile. In contrast, no correlation was found between V_{\text{max}} and blunting of the profile (V_{\text{max}}/V_{\text{mean}}).

A feature of all velocity profiles was a considerable scatter of the data points at each relative radial position. In general, the scatter was greater near the wall than in the center of the vessel, as is apparent in Figure 4C. The scatter is caused on the one hand by systematic changes in arteriolar velocity, for instance due to variations in cardiac output, and on the other hand by random variations in platelet velocity. In the same TV picture, differences in velocity could be measured for platelets flowing at an almost identical relative radial position. This effect was most pronounced for platelets flowing near the vessel wall. Variations in displacement of platelets which were sliding in apparent contact along the same wall but at different downstream positions were found as large as about 20%. In the center of the vessel variations could be found as large as about 6%.

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Leukocyte and Red Blood Cell Velocities

The velocities of the few leukocytes observed during determination of a platelet velocity profile, generally fell within the range of platelet velocities as measured at the concomitant relative radial positions. Examples are presented in the Figures 2, 3A, 4A, and 5, in which the velocity of a white blood cell is indicated by an open square. The total number of leukocytes observed during determination of the platelet velocity profiles was 24.

In Figures 5A and B examples are shown of a red blood cell velocity profile. The velocity of a red blood cell is indicated by a cross. For comparison, the corresponding platelet velocity profile, obtained in the same vessel but several minutes later, is presented as well. The profiles presented in the Figures 5A and B were assessed in different arterioles; the profiles in Figure 5A during the diastolic phase and the profiles in Figure 5B during the systolic phase. It is clear from the Figures 5A and B that the velocities of the red blood cells fell within the range of platelet velocities, when measured at the concomitant relative radial positions, despite the fact that no correction was made for slight changes in velocity that might have occurred between the moment of assessment of the red blood cell and platelet velocity profiles. In another experiment, the velocity in the vessel did change considerably during the interval between the assessment of the red blood cell and the platelet velocity profile (from an average centerline velocity of about 23 mm/sec to about 14.5 mm/sec). However, following normalization of each profile with respect to its regression fit centerline velocity, the red blood cell velocities fell again within the envelope of the platelet velocity profile.

A fit of the red blood cell velocity profiles with Equation 2 yielded a 3-5 times higher standard error of parameter a than in case of the platelet profiles. This inaccuracy is caused by the fact that the centers of gravity of the red blood cells, in contrast to those of the platelets, cannot come closer to the vessel wall than about 2.5 \( \mu m \). When the red blood cell profiles were fit with the values of a and K obtained from the concomitant platelet profiles, nonsystematic patterns of residuals were obtained. Therefore, the results of these experiments indicate that, within the region of the cross-sectional area of the vessel where the centers of gravity of both red blood cells and platelets are present, the shape of the velocity profile of the red blood cells is similar to that of the platelets.

Discussion

In the present study velocity profiles have been determined in arterioles, using blood cells which were preferentially labelled with a fluorescent dye as natural markers of flow. Compared to high speed cinematography and bright field microscopy, as used in earlier studies, detection of the cells of interest has considerably improved with the fluorescence technique employed in this study. In addition, the smallest blood cell, the platelet, can be used as a marker of flow, providing for the first time in vivo quantitative information about the shape of the velocity profiles in microvessels. Because platelet velocities can be measured close to the wall (about 0.5 \( \mu m \)), and this information determines to a large extent the shape of the profile, profiles as assessed from the velocity distributions of platelets are more reliable than those obtained from the velocity distributions of red blood cells. Red blood cells cannot come closer to the wall with their center of gravity than about 2.5 \( \mu m \).

The study shows that in arterioles the velocity profiles of blood platelets differ significantly from a parabolic velocity distribution, both in diastole and systole. The profiles are flattened as compared to a parabola. In addition, the study indicates that the shape of the velocity profiles of red blood cells and blood platelets is similar within the region where the centers of gravity of both cells are present. The velocities of the few white blood cells which were observed during determination of the platelet velocity profiles, fell within the range of platelet velocities as measured at a similar radial position within the vessel. The latter two findings suggest that differences in shape, if any, of the velocity distributions of the different blood cells are limited. In general, the velocity of a particle in flowing blood will reflect the velocity of the surrounding fluid. Therefore, it seems reasonable not only to regard the shape of the platelet velocity profiles as representative of that of the plasma, but also as representative of that of the other blood cells. It should be pointed out, however, that this does not imply that the average flow velocity of the different blood components is similar, because this velocity also depends on their distribution over the cross-sectional area of the vessel. For instance, the average flow velocity of the blood platelets will be less than that of the red blood cells, since in
arterioles the concentration of blood platelets is higher near the wall than in the center of the vessel, in contrast to the distribution of the red blood cells.

In this study velocity profiles were constructed by measuring the velocity of cells flowing within an optical section around the median plane of the vessel. To evaluate whether the flattening of the profiles was caused by the finite depth of the optical section, the influence of the section depth on the shape of the profile was calculated. Assuming a parabolic velocity profile, the mean velocity in the optical section at different radial positions was calculated (see "Appendix") for a relative section depth, i.e., the section depth as compared to the vessel diameter, of 0.2, 0.4, 0.6, and 1. The results as obtained for a relative section depth of 0.2 and 0.4 μm are presented in Figure 6. These relative section depths correspond to the approximate section depths in the case of platelets (5 μm) and red blood cells (10 μm), respectively, in a vessel with a diameter of 25 μm.

As indicated by the data presented in Figure 6A, the mean velocity in the optical section at each radial position decreased with an increase in relative section depth. The maximal velocity as a percentage of the control value was 99, 95, 88, and 67% for a relative section depth of 0.2, 0.4, 0.6, and 1, respectively. However, the shape of the profiles was hardly influenced as is shown in Figure 6B. In this figure the normalized profiles, as found with relative section depths of 0, 0.2, and 0.4 are presented. In the case of a relative section depth of 1, i.e., equal to the vessel diameter, the shape of the profile was a parabola.

The development of the laminar flow profile, because an incomplete development of the laminar flow profile, because all measuring sites were at least six vessel diameters downstream from a side branch and in the microcirculation the entrance length will only be about one vessel diameter.1 Curves immediately upstream or downstream of the site of measurement could not always be avoided, which might cause shifting of the top of the profile.2 It is unlikely, however, that such shifts have caused the flattening of the profiles in the present study. First, blunting of the profiles was observed in straight vessel segments without nearby curves. Second, in all experiments the top of the velocity profile was positioned close to the vessel center, indicating that in this study the influence of curves on the shape of the profile, if any, was small.

![Figure 6](http://circres.ahajournals.org/content/59/5/512/F6.large.jpg)

**Figure 6.** Influence of the depth of the optical section (d) as compared to the vessel diameter (D), i.e., the relative section depth (d/D), on the mean velocity in the optical section at each relative radial position (A), and on the shape of the velocity profile (B), assuming a parabolic velocity profile. For calculation see Appendix. For each value of d/D, the mean velocity in the optical section at different radial positions was normalized with respect to the centerline velocity of the parabola (curve d/D = 0) in A, and with respect to the centerline velocity of its own velocity profile in B. Upper curve in B is for d/D = 0, lower curve for d/D = 0.4.
Pulsatile flow could be observed in the arterioles studied. In large vessels acceleration and deceleration of the blood during the cardiac cycle contribute to the marked deviations of the velocity profile from a parabola. However, in arterioles the influence of inertia will be negligibly small, and hence pulsatility cannot be the cause of the flattening of the profiles. In addition, in glass tubes of 40 μm in diameter no difference in velocity profile was found during pulsatile or steady flow.

Analysis of the present data shows that a proper line through the experimental points could only be obtained when the fits were not forced through zero velocity at the vessel wall, yielding a positive intercept of the fits with the wall. Platelet velocities could be measured as close to the wall as about 0.5 μm. Assuming zero velocity at the wall (no-slip condition), the results of the present study indicate the existence of a small region with a steep velocity gradient at the wall. Hence, the apparent viscosity of the thin layer of fluid at the wall differs considerably from the apparent viscosity of the fluid in the remainder of the vessel, suggesting that it will be difficult to describe blood flow in small vessels with a one-fluid model, i.e., a theory in which the flow properties of blood are assumed to be uniform over the whole cross-sectional area of the vessel.

The difference in apparent viscosity between the thin layer of fluid at the wall and the fluid in the remainder of the vessel, is caused, among others, by a difference in concentration of the red blood cells. Because the volume fraction of the red blood cells is far greater than that of platelets or white blood cells, the flow characteristics of blood are mainly determined by the hematocrit. As mentioned, the centers of gravity of the red blood cells could not come closer to the vessel wall than about 2.5 μm. Due to this wall exclusion effect the hematocrit of the thin layer of fluid at the wall will be less than that of the fluid in the remainder of the vessel. Inward migration of the red blood cells might have added to this effect. It should be pointed out, however, that this was not a cell-free layer, at least in the mesenteric arterioles investigated. All three types of blood cells could be observed in apparent contact with the wall.

The existence of a layer of fluid at the vessel wall with a relatively low apparent viscosity, is responsible for the flattening of the velocity profile because of the relatively high velocities near the wall. On the other hand, in the center of the vessel the velocities were lower than expected on the basis of a parabola that was not forced through zero velocity at the wall, indicating that an additional effect has contributed to the blunting of the profiles. At low shear rates, for instance, in the center of the vessel, the presence of red blood cells causes a marked increase in the apparent viscosity, which will lead to a flattening of the velocity profile. It may even lead to plug flow in this region. However, in most experiments differences in velocity could be observed between the vessel wall and the center of the vessel, or close to it, indicating the presence of a limited region of plug flow, if any, in these vessels. Moreover, the variations in velocity at a similar radial position, as observed in the present study in one TV picture, suggest that even in a region of plug flow small differences in velocity will be present due to interactions between neighboring blood cells.

In conclusion: The data in the present study shows that the velocity profiles, as assessed in mesenteric arterioles in vivo, are flattened as compared to a parabola, both in systole and diastole.

Appendix: Calculation of the Influence of the Depth of the Optical Section on the Shape of the Velocity Profile and the Scatter of the Data Points

A cartesian coordinate system \((x,y,z)\) is constructed with the \(x\)-axis along the optical axis, the \(y\)-axis along the radius of the vessel and the \(z\)-axis along the axis of flow. The origin of this coordinate system is positioned in the center of the vessel. Assuming a parabolic velocity profile in the vessel, the velocity at a certain point, \(V(x,y)\), is given by

\[
V(x,y) = V_{\text{max}} \left[1 - \frac{x^2 + y^2}{R^2}\right], \quad (A1)
\]

where \(V_{\text{max}}\) = the centerline velocity, \(R\) = the radius of the vessel.

An optical section with a depth \(d\) is located around the median plane of the vessel. Within the optical section, the mean velocity at a certain radial position, \(\bar{v}_d(y)\), is given by

\[
\bar{v}_d(y) = \frac{2}{d} \int_0^{d^2} \frac{V_{\text{max}} \left[1 - \frac{x^2 + y^2}{R^2}\right]}{x^2 + y^2} \, dx
\]

for \(|y| \leq \sqrt{R^2 - \frac{d^2}{4}}\), and

\[
\bar{v}_d(y) = \frac{V_{\text{max}}}{\sqrt{R^2 - y^2}} \int_0^{\sqrt{R^2 - y^2}} \left(1 - \frac{x^2 + y^2}{R^2}\right) \, dx
\]

for \(\sqrt{R^2 - \frac{d^2}{4}} \leq |y| \leq R\) \( (A2)\)

Vertical stripes denote absolute value. Solving Equation \(A2\) yields:

\[
\bar{v}_d(y) = V_{\text{max}} \left[1 - \frac{y^2}{R^2} - \frac{d^2}{12R^2}\right]
\]

for \(|y| \leq \sqrt{R^2 - \frac{d^2}{4}}\), and

\[
\bar{v}_d(y) = V_{\text{max}} \left[\frac{2}{3} - \frac{2y^2}{3R^2}\right]
\]

for \(\sqrt{R^2 - \frac{d^2}{4}} \leq |y| \leq R\) \( (A3)\)

The maximal and minimal velocity in the optical section at a certain radial position can be obtained from Equation \(A1\) for \(x = 0\) and \(x = 1/2d\), respectively.
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