

THE RELATIONSHIP BETWEEN LEUKOCYTE AND ERYTHROCYTE VELOCITY
IN ARTERIOLES

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Introduction

Geometrical, rheological and functional differences between erythrocytes and leukocytes may give rise to differences in the in vivo flow dynamics of these two cellular components of blood. One index which may shed light on specific differences, if present, is the ratio of leukocyte velocity (V_{wbc}) to erythrocyte velocity (V_{rbc}) in small arterioles. No systematic study of the relationship between these two quantities or the possible exploitation of a demonstrated correlation between them has been reported. Further, when such measurements are made simultaneously in vessels not significantly larger than leukocyte dimensions the deviation of V_{wbc}/V_{rbc} from unity might provide a measure of an in vivo flow variance attributable to some differential aspect between the two cellular components. The purpose of the work reported here was to investigate the relationship between V_{rbc} and V_{wbc} in small arterioles and to establish base line data on the normally occurring statistical distribution of the ratio of these two quantities.

Methods

Eight female hamsters (110-140 gms) anesthetized with pentobarbitol (.06mg/gm IP) were prepared for microscopic observation of the cheekpouch vasculature which was exposed and everted using standard techniques(1). An arteriole not greater than 15um in diameter was selected for study and

Vrbc determined continuously for a period of 60 minutes using the photo-optic/crosscorrelation method(2,3). Simultaneously the microscopic image of the arteriole was recorded on video tape at an optical magnification of approximately 720 using a nuvicon TV camera and a BG/28 glass filter chosen to enhance the contrast of the leukocytes. Vwbc was determined by sensing the transit of each cell over a known axial distance using two cursors inserted into the video image of the arteriole. The electronic output of each cursor was displayed on a chart recorder and thereby permitted the determination of transit time and hence the calculation of Vwbc.

Each 60 minute experiment was divided into 240 consecutive intervals of 15 seconds duration. For each interval the number of leukocytes (wbc flux) and their velocity were determined. The average Vwbc in each interval was compared with the average Vrbc over the same interval by forming the ratio Vwbc/Vrbc. Statistical techniques were used to evaluate the distribution of this ratio within single and multiple intervals.

Results

One of the principle results of the present study is the demonstration of a very small difference between red blood cell velocity (Vrbc) and white blood cell velocity (Vwbc) simultaneously determined in arterioles with diameters ranging from 6.8 to 13.5 μm . Based on 20,485 separate Vwbc measurements the average ratio Vwbc/Vrbc determined for all vessels was found to be 0.95 ± 0.06 (mean \pm SD) as summarized in Table 1. This close agreement between Vwbc and Vrbc persisted despite an eight-fold variation in Vrbc and more than a three-fold variation in systemic wbc count.

TABLE 1. DATA SUMMARY - PARAMETERS AND RANGES

Diameter(μm)	Vrbc(mm/sec)	WBC Count	# of WBCs	Vwbc/Vrbc
6.5-13.5	0.30-2.40	2600-7900	20,485	0.95 ± 0.06

As an illustration of the relative constancy of the V_{wbc}/V_{rbc} ratio, Figure 1 summarizes the data obtained in one experiment from a 12.9 μm diameter arteriole in which V_{rbc} varied from 0.4 to 1.8 mm/sec over the course of the measurement period (60 mins).

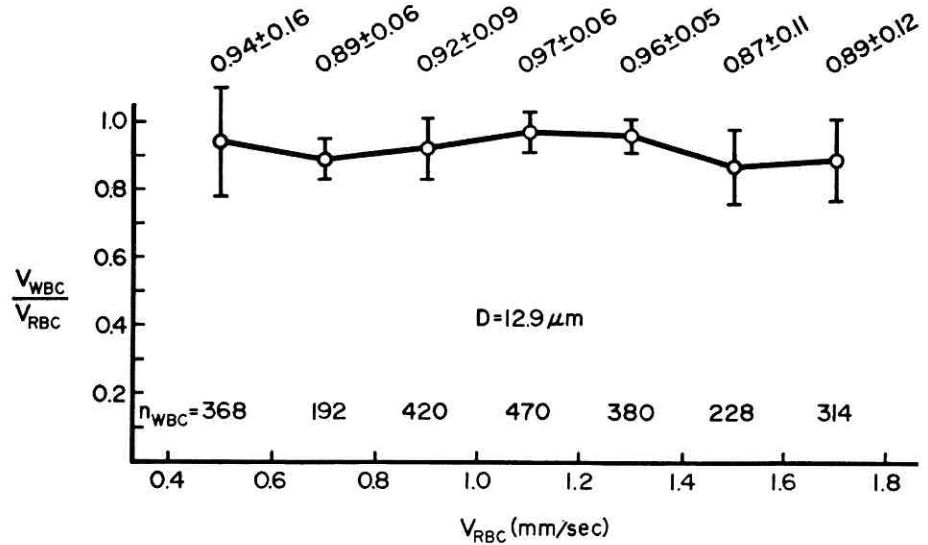


FIGURE 1. Ratio of V_{wbc} to V_{rbc} determined over the course of 60 minutes in an arteriole with large excursions in blood velocity. Data are for $V_{rbc} \pm 0.10$ mm/sec. Ratio = mean \pm SD.

The velocity variations occurred spontaneously and without change in the diameter of the observed vessel. Utilizing this fortuitous spontaneous velocity change and by choosing contiguous V_{rbc} bins of 0.2 mm/sec width, one could determine the number of wbc's measured (n) within each quantized velocity range. For each group the average V_{wbc}/V_{rbc} ratio so calculated could then be expressed as shown in Figure 1. As may readily be seen, V_{wbc}/V_{rbc} was not less than 0.87, nor after calculation was there any statistical difference between velocity ratios as determined by analysis of variance. Further results demonstrating the close correspondence between V_{rbc} and V_{wbc} are shown in

Figure 2, which summarizes the data of four separate experiments.

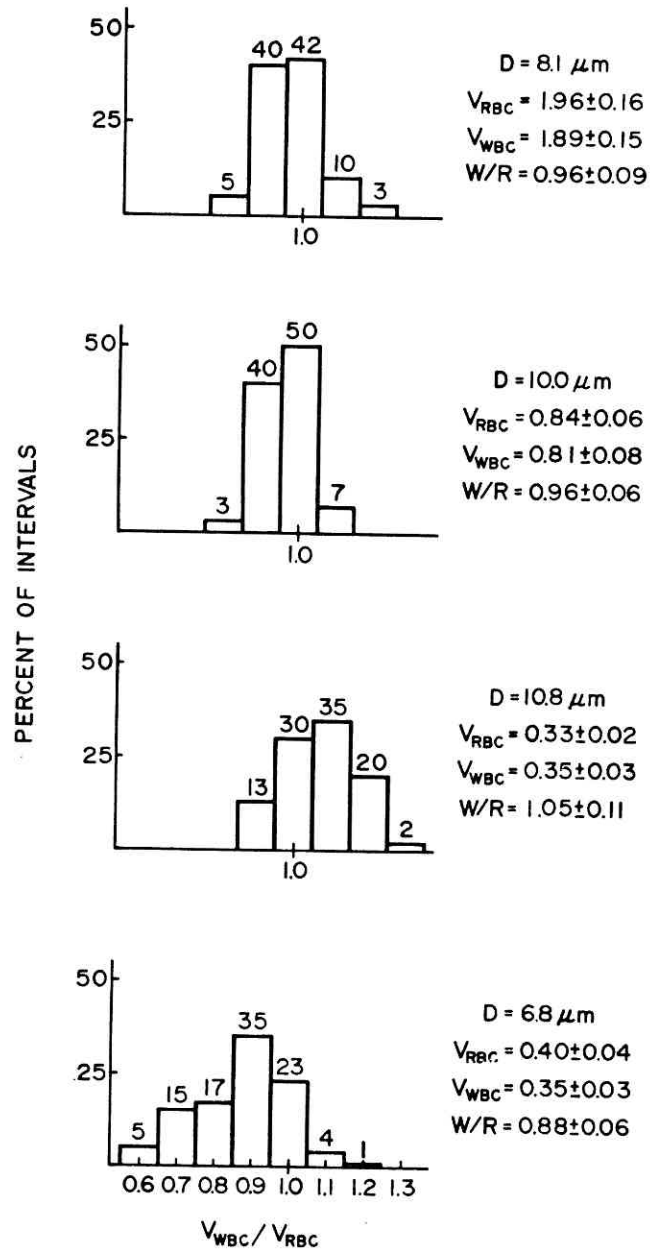


FIGURE 2. Frequency distribution of V_{wbc}/V_{rbc} for widely varying hemodynamic conditions.

The cases selected for presentation in Figure 2 were chosen so as to well illustrate the distribution of V_{wbc}/V_{rbc} for fairly wide hemodynamic and arteriole diameter differences.

Each histogram shows the percentage of the 240 separate 15-second intervals in which the V_{wbc}/V_{rbc} ratio was between the indicated ranges. The top panel, which illustrates a high V_{rbc} and intermediate diameter case, shows for example that in 82% of the intervals the V_{wbc}/V_{rbc} ratio was between 0.85 and 1.05. The middle two panels, which are for data obtained from similar diameter arterioles but significantly different blood velocities, are similarly tightly distributed but with mean values slightly below and above the unity ratio.

The bottom panel shows the distribution associated with a low blood velocity occurring in the smallest diameter arteriole studied. The V_{wbc}/V_{rbc} ratio in this case is more widely distributed than the previous cases and though skewed more toward lower values the mean ratio of 0.88 still represents a V_{wbc}/V_{rbc} ratio only slightly deviant from unity.

Discussion

Insight into capillary and post-capillary wbc intravascular processes and its relationship to microvascular hemodynamics continue to emerge(4-8). Information and understanding of wbc dynamics in arterioles is less well developed. In medium size arterioles the dependence of wbc-vessel wall interaction phenomena on blood velocity (9,10), transient blood stasis (11), focal tissue trauma (12), and microvascular hemodynamics (13) has been clarified but little data on events in arterioles less than about 15 μ m is available (14,15). This problem has been addressed in the present work with the main parameter of interest being the ratio of white blood cell velocity (V_{wbc}) to red blood cell velocity (V_{rbc}).

The data obtained from over 20,000 measurements of white blood cell velocity in terminal arterioles has shown that on the average V_{wbc} is in fact slightly less than the

value of red blood cell velocity determined using now standard cross-correlation techniques. The value for V_{wbc}/V_{rbc} of 0.95 ± 0.06 obtained as the average of all data suggests that the hemodynamic impact of the white cell under normal conditions is minimal in the arteriole vessel size range studied. However it must not be inferred that this cellular component of blood is without hemodynamic effects. Firstly, the present method determines the V_{wbc}/V_{rbc} ratio with the wbc's present in the vessel in which the measurement is made. Even though no significant deviation in this ratio from its mean value was found as a function of the wbc flux vessel (5-50 cells/min) the possibility that V_{rbc} would be higher in wbc free vessels (and the converse) cannot be ruled out. Further, significant flow retarding processes may well be found in smaller diameter vessels; at critical pre-capillary branch points; under conditions of reduced pressure gradient; or under conditions of enhanced wbc vessel wall adherence.

When, as in the present case, V_{wbc} is determined in the absence of these possible complicating factors it can be utilized as an effective index of the absolute value of mean red blood cell velocity with a calibration factor to account for the small deviation found.

The use of wbc tracking for the subjective evaluation of retinal blood flow via the blue field entoptic method (16) has already been utilized. The present work represents the first analytical approach known to the author to in fact establish its validity provided that the retinal capillaries are greater than a critical diameter. The results of the present work also show that under conditions in which V_{rbc} cannot be determined directly, V_{wbc} will yield adequate values for the absolute value of blood velocity and together with diameter measurements allow calculation of blood flow. Of special interest in this regard is when fluorescent microscopy is utilized to study in vivo wbc dynamics in the microvasculature. The low light levels available do not readily permit V_{rbc} to be determined using correlation

methods. This previous limitation can now be overcome and simultaneous information on wbc dynamics and hemodynamics can be reliably obtained.

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