

Leukocyte Distribution to Arteriolar Branches: Dependence on Microvascular Blood Flow

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Leukocyte distribution to arteriolar branches in the hamster cheek pouch microvasculature was studied by rendering the cells fluorescent via a constant intravenous infusion of acridine orange. A principal objective was to determine the major factors influencing the partitioning of the cellular flux between daughter branches at arteriolar branch points with internal diameters permitting single-file leukocyte flow. Leukocyte flux determinations (cells/sec) and individual cell velocities were made for over 8000 cells at 13 branch sites in 10 animals. The results indicate that for the branch flow ratios of 0.3 to 0.7 encountered in the present experiments, the predominant factor influencing the average leukocyte flux is the branch blood flow. Branch fractional flux dependence on fractional blood flow was analyzed using linear and nonlinear models. A linear characterization provided an excellent fit and description of the functional relationship but a slight nonlinear component was also predicted by the nonlinear sigmoidal model which also fit the data well. Because of the small degree of predicted nonlinearity and the overlap of the standard deviations of the parameter estimates, we were not able to statistically decide on which model, and hence which functional relationship, is operative. Thus, the answer to the question regarding the presence of any preferential distribution is inconclusive. However, the data and analyses suggest that if preferential distribution is operative, its magnitude over the flow range and conditions here studied is small, and that for many purposes the relationship between the *in vivo* average fractional cell flux and blood flow may be viewed as linear. © 1985 Academic Press, Inc.

INTRODUCTION

Under suitable conditions direct *in vivo* microscopic observation of the microcirculation permits visualization of circulating leukocytes in both arterioles and venules. In spite of recent inroads in characterizing the *in vivo* dynamics of these cellular blood components (Atherton and Born, 1973; Bagge and Branemark, 1977; Mayrovitz *et al.*, 1977; Schmid-Schonbein *et al.*, 1980b) little is known concerning the factors which determine the way in which leukocytes in a parent arteriole distribute to its branches. At such an arteriolar branch point the bulk blood flow distributes into the two diverging branches in an amount which is inversely related to the vascular resistance of the two pathways. On this basis it may be argued that the number of white blood cells (WBC) per unit time entering each branch (WBC flux) will be proportional to the bulk blood flow in that branch and the WBC concentration in the parent vessel. Though at first glance this hypothesis seems reasonable for vessels significantly larger than the WBC, its applicability to terminal arterioles is still open. Polymorphonuclear

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granulocytes (PMN), which were of prime interest in this study are classically thought to have diameters which range from 10 to 15 μm (Wintrobe, 1967; Williams *et al.*, 1972). Recent data suggest a somewhat smaller *in vivo* diameter of 6 to 9 μm (Schmid-Schonbein *et al.* 1980a). Since the WBC size is comparable to the terminal arteriole diameter, it may be that the WBC distribution at branch points will depend on the local vessel diameter, branching angle, and other local factors. This view is advocated by some workers. On the basis of hydrodynamic theory and *in vitro* models (Fung, 1973; Yen and Fung, 1978) it has been postulated that the distribution of red blood cells at arteriolar branch points preferentially (strong nonlinear dependence) favors the movement of RBCs into the branches with the greater flow. Yen and Fung (1978) show this to occur in an *in vitro* simulation provided that a critical velocity ratio is present. The logical extension of this theory to the *in vivo* situation may be that at some critical flow ratio all cells will move into the vessel with a greater flow. Limited *in vivo* data (Klitzman and Johnson, 1982) has shown a slight, but statistically significant, preferential distribution at arteriolar levels for erythrocytes. As specifically related to the distribution of WBC, Bagge and Branemark (1977) have speculated on the presence of physiological shunting of leukocytes through the microcirculation in wider channels. Further, Schmid-Schonbein *et al.* (1980b) have reported that in narrow vessels leukocytes tend to follow the vessels with the fastest flow. These conclusions, however, were based on measurements of a very small number of leukocytes. The question of which factors predominantly influence the distribution of cellular components at arteriolar branch points, especially with regard to the distribution of leukocytes, remains open. It is the purpose of this report to provide further information pertinent to this controversial issue.

METHODS

Animal Preparation

Syrian golden hamsters of both sexes weighing 90–120 g supplied by Charles River Laboratory were used in this study. Prior to surgery each animal was anesthetized with pentobarbital (0.6 ml/100 g body wt ip). Additional doses of anesthesia were given sequentially as required. The animal was secured to a specially designed observation board in a supine position and core body temperature maintained by a heating mat throughout the surgical procedure as well as during the observation period to follow. A tracheal cannula (PE-200) was inserted to ensure patent airway during the experiment. The left femoral vein was cannulated (PE-20 tubing pulled to the desired diameter) and the patency of the femoral vein was maintained with heparinized Normosol. A single-layer cheek pouch preparation was performed according to the basic technique first described by Fulton *et al.* (1946) and later by Duling (1973).

Experimental Setup

Following the surgical procedure the animal was transferred to the stage of a Laborlux 12 HL Leitz fluorescence microscope equipped with a 12 V, 100-W tungsten halogen lamp for transillumination and a 50-W ultrahigh-pressure mercury lamp used as an epiillumination system.

The optical system consisted of a 50 \times water immersion objective with a

numerical aperture of 1.00. Occasionally a $32\times$ long-working-distance dry objective was used to get a larger field of view. Oculars used during the course of the experiment were $16\times$ and $10\times$, wide-field lenses. A $5\times$ or $10\times$ ocular was used in a trinocular tube of the microscope for the purpose of obtaining video information. To this end an MTI-65 low-light level TV camera was attached to the trinocular tube with a specially designed adapter which permitted rotating the camera and its projected image 360 degrees. The video image obtained from the low-light level TV camera was recorded on a JVC video recorder and displayed on a 19 in. Concord TV monitor.

Vessel Selection

Arteriolar junctions suitable for study were chosen utilizing the transillumination system according to the following criteria: (1) the feeding vessel and the two diverging branches were in the same focal plane; (2) all three vessels were within a diameter range of $5\text{--}20\ \mu\text{m}$ as determined by measurements of the internal diameter at several sites along the vessel length; (3) each of the vessels chosen for observation showed single-file leukocyte flow.

Experimental Protocol

Acridine orange ($50\ \mu\text{g}/\text{ml}$ filtered and pH balanced to 7.4, Aldrich Co.) was infused via the cannulated femoral vein at a constant rate of $0.00866\ \text{ml}/\text{min}$ (Harvard apparatus pump, Model 902). Leukocytes stained with the acridine orange and brilliantly fluorescing, were observed traveling along the feeding vessel and through them to diverging branches. Data was recorded through the video system for periods of time not less than 10 min. A total of 10 animals were used in this study and in 3 of these, 2 branch points were so studied thus giving a total number of 13 branch points.

Data Acquisition

The following parameters were measured; feeding vessel diameter, the diameters of the two branches, the velocity of each individual WBC, WBC flux (number of WBCs per unit time) at each branch as well as the branching angle of each bifurcation point.

Diameters of each vessel were measured at several sites along the vessel through a reticule in the $16\times$ ocular calibrated previously with a stage micrometer ($10\ \mu\text{m}/\text{division}$, American Optical).

The velocity of each individual leukocyte was found as follows. Two electronic cursors were superimposed on the recorded video image of the vessel, one upstream and the other downstream. The cursors were sensitive to changes in optical density, which changed rapidly as each fluorescent WBC passed by. The change in optical density was detected and transduced into an electronic pulse which after proper amplification with an ac-coupled differential amplifier (Techtronics, AM 502) was fed into a dual channel chart recorder (MFE 1200). The known axial distance between the cursors divided by the transit time of each cell was used to calculate the velocity of individual leukocytes. Blood flow was calculated in each vessel as the product of WBC velocity and vessel cross-sectional area. Empirical correction factors for the calculation of *in vivo* bulk velocity and blood flow were not used. It was felt that in spite of recent progress

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in this area (see for example, Starr and Frasher, 1975; Lipowsky and Zweifach, 1978; Albrecht *et al.*, 1979; Schmid-Schonbein *et al.*, 1980b) a sufficient number of unresolved issues remain with regard to the proper factor(s) for *in vivo* use.

The WBC flux was obtained in a similar manner; the two cursors were positioned, one on each branch. A series of pulses obtained for each branch described the temporal distribution of the WBC arrival time at each branch.

The branching angles of each arteriolar bifurcation were measured either by positioning a protractor on the still televised image of the junction point, or by observing the branch point through a protractor reticle eyepiece in real time.

RESULTS

General Properties of Vessels Studied

Table 1 provides a summary of the average values and ranges obtained for the geometric and dynamic parameters. In the summary data below all values are presented as means \pm 1 SD.

Vessel diameter. Thirteen precapillary terminal arteriolar branch points were studied in the cheek pouch of 10 male and female golden hamsters. Diameters of the vessels, measured between the innermost boundary of the endothelial layer, ranged from 6.4 to 20 μm . The mean diameter of the parent feeding vessel

TABLE 1
SUMMARY OF GEOMETRIC AND DYNAMIC
PARAMETERS

	Mean	Range
Arteriolar		
Diameter (μm)		
D_0^{*a}	14.0	8.8-20
$D_{1,2}$	11.5	6.4-20
Branch Angle (degrees)		
A_1	46	1-116
A_2	56	32-94
Velocity ($\mu\text{m}/\text{sec}$)		
V_0	992	229-2838
$V_{1,2}$	729	113-2014
Flow ($\text{ml}/\text{sec} \times 10^{-8}$)		
Q_0	14.2	3.5-38.3
$Q_{1,2}$	7.0	1.7-26.7
WBC flux (cells/sec)		
F_1	0.95	0.3-3.4
F_2	0.45	0.1-1.8

^a The subscripts 0, 1, and 2 refer to the parent vessel, the branch with the greater WBC flux and the branch with the lesser WBC flux, respectively. When no distinction between branches is made the designation is 1,2.

(D_0) was $14 \pm 3.37 \mu\text{m}$. The ratio of daughter vessel diameter to parent vessel diameter averaged 0.82 ± 0.11 . The mean diameter of the branch that received the greater fraction of cells (D_1) was $12.1 \pm 4.18 \mu\text{m}$ as compared to the mean diameter of the vessel with the lesser flux (D_2), $11.0 \pm 2.53 \mu\text{m}$. There was no significant difference between diameters of these two groups of vessels ($P = 0.381$, Student's t test).

Branching angle. Total branching angle ($A_1 + A_2$) varied between 57° and 191° . The mean angle for the vessel with the greater flux (A_1) was $46 \pm 37^\circ$ and $56 \pm 21^\circ$ for the vessel that received the lesser flux (A_2). No significant differences were found between the branching angle of these two groups ($P = 0.451$, Student's t test).

Blood velocity. The mean blood velocity in the feeding vessel (V_0) averaged $992 \pm 659 \mu\text{m}/\text{sec}$. The mean velocity in the vessel with the greater flux (V_1) was found to be $816 \pm 523 \mu\text{m}/\text{sec}$ as compared to the vessel with the lesser flux (V_2), $641 \pm 383 \mu\text{m}/\text{sec}$, and the velocity ratio was 1.44 ± 0.85 . The data revealed more than a 17-fold spread in branch velocity values (113 – $2014 \mu\text{m}/\text{sec}$) across experiments with a small variance within each experiment.

Blood flow. Blood flow in each vessel was calculated as the product of WBC velocity and vessel cross-sectional area. Flow (mean and standard deviation), in the feeding vessel (Q_0) was $14.2 \pm 9.8 \times 10^{-8} \text{ ml}/\text{sec}$. Flow in the branch with the greater flux (Q_1), averaged $8.84 \pm 6.9 \times 10^{-8} \text{ ml}/\text{sec}$ and the flow in the branch with lesser flux (Q_2), was $5.4 \pm 3.2 \times 10^{-8} \text{ ml}/\text{sec}$.

Flux rate and total number of cells. The total number of WBCs detected was 8222. The mean flux rate ranged between 0.17 and 3.4 cell/sec. The mean flux rate in the vessel with the greater flux ($N = 13$) averaged $0.95 \pm 0.86 \text{ cells}/\text{sec}$ and the flux in its paired counterpart ($N = 13$) averaged $0.45 \pm 0.31 \text{ cell}/\text{sec}$.

WBC Flux Dependence on Blood Flow

Figure 1 shows the relationships between the measured WBC flux and the blood flow in each of the arterioles studied. Each data point represents the mean obtained for each vessel over the total observation time which ranged from 10 to 15 min. A regression analysis using each paired flux-flow value yielded the regression equation $F = 0.099Q - 0.037$ ($r = 0.910$, $P < 0.001$) in which flux is

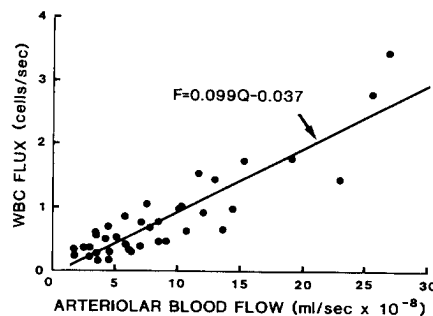


FIG. 1. White blood cell flux as a function of arteriolar blood flow. Data (closed circles) was obtained from 38 arterioles having diameters ranging from 6.4 to $20 \mu\text{m}$. Each data point represents the mean obtained from each vessel over a time period of 10 to 15 min. The solid line is the standard linear regression equation relating WBC flux (F) to blood flow (Q), $r = 0.910$, $P < 0.001$.

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the number of observed cells per second and the flow is in units of ml/sec $\times 10^{-8}$.

The solid line drawn through the data in the figure is a graph of this regression equation. The close dependence of WBC flux on flow is clearly seen from this data and is quite independent of specific arteriolar branch, vessel diameter, or branching angle. Separate regression analyses of these parameters as they relate to WBC flux indeed fail to show any significant correlation. Further, since the data contained in Fig. 1 is a composite across experiments, it implies that in spite of probable variations in total WBC counts (not measured) between animals, the closely linear dependence of WBC flux on blood flow still obtains.

WBC Flux Distribution at Branch Points

Fractional flow dependence. To examine more closely factors which may be operative in determining the fractional distribution of the WBCs between paired daughter branches, analysis of parameter ratios were used. The major point of interest was to evaluate the relationship between the fractional flux ratio, F_R (WBC flux in a branch/total WBC flux) and the fractional flow ratio, Q_R (blood flow in that branch/total blood flow).

One of the principal concerns of this analysis was to try to determine if we could ascertain whether WBC were distributed preferentially to branches with a greater amount of blood flow. If no such preferential distribution were operative then F_R would be anticipated to be directly proportional to Q_R . Under these conditions the F_R - Q_R relationship would be expected to conform to the linear model

$$\text{Model A} \quad F_R = aQ_R.$$

At the other extreme, if a complete preferentiality were present, then in the limit all WBCs would go to the branch with the greater flow. As indicated by Schmid-Schonbein and co-workers (1980b) under these extreme conditions the relationship between F_R and Q_R would be a step function, with F_R being zero in the interval for which $Q_R < 0.5$ and equal to unity in the interval for which $Q_R > 0.5$. However, as pointed out by Klitzman and Johnson (1982), when preferential distributions of the type being discussed are present, but not completely dominant, then one may anticipate a sigmoidal relationship between F_R and Q_R which includes the F_R, Q_R points (0,0), (0.5,0.5), (1,1) and which lies between the linear and step function models. This sigmoidal model may be characterized as

$$\text{Model B} \quad F_R = \{[(1 - Q_R)/Q_R]^b + 1\}^{-1}.$$

It may be noted that if in model B the parameter b is unity, then model B reduces to model A with the parameter $a = 1$, i.e., no preferential distribution. Using all paired experimentally determined F_R - Q_R values we have estimated the parameter values in models A and B using least-squares error analyses. For completeness we have also evaluated the standard linear regression model $F_R = a_1Q_R + b_1$ and the nonlinear parabolic model $F_R = a_2Q_R^b$. The resulting parameter values and their asymptotic standard deviations are summarized as follows: model A, $a = 1.01 \pm 0.04$; model B, $b = 1.14 \pm 0.18$; standard linear model, $a_1 = 1.12$

parent vessel that received 1 to the mean. There was no difference between vessels ($P =$

between 57° and $46 \pm 37^\circ$ and that differences were not significant (Student's

V_0) averaged after flux (V_1) with the lesser of 85. The data were $13-2014 \mu\text{m}^2/\text{min}$.

product of WBC flux (deviation), in that branch with the greater flow in the

was detected was the mean flux of 0.86 cells/sec and 0.31 cell/sec.

flux and the ratio of the mean fluxes yielded the ratio of which flux is

and circles) was the point represents is the standard deviation (0.001).

± 0.16 and $b_1 = -0.06 \pm 0.08$; parabolic model, $a_2 = 1.09 \pm 0.12$ and $\beta = 1.13 \pm 0.18$. The regression coefficient for the standard linear model was found to be $r = 0.870$, $r < 0.001$. In Fig. 2, the predicted functional relationships between F_R and Q_R according to models A and B are graphed along with the experimentally determined values of F_R and Q_R .

Visual inspection of Fig. 2 suggests that over the range for which experimental data is available (Q_R range approximately 0.3 to 0.7) either model may be used to represent the functional relationship between F_R and Q_R . Because of the small degree of predicted nonlinearity ($b = 1.14 \pm 0.18$) and the associated overlap of the standard deviations between this nonlinear parameter and the linear model parameter ($a = 1.01 \pm 0.04$) we are not able to statistically separate them. Thus the results as to whether any preferential distribution is present is inconclusive. Similar comparisons using the other models are equally inconclusive regarding this point. However, the data and analyses suggest that if preferential distribution is operative, its magnitude over the flow range and conditions here studied is small, and that for a variety of purposes the F_R - Q_R relationship may be approximated as a linear one.

Velocity ratio dependence. Further analyses were carried out to examine the dependence of the WBC flux distribution on cell velocity in the branches. In Table 2 the average flux and velocity ratios for each paired branch studied are tabulated. Also included are the vessel diameter ratios and the ratio of cell to vessel diameter assuming an average WBC diameter of $8 \mu\text{m}$. The convention that the subscript 1 designates the branch with the greater flux is maintained. The experimental data, F_1/F_2 vs V_1/V_2 , is plotted in Fig. 3. These data have been subjected to least-squares error analyses according to the following models:

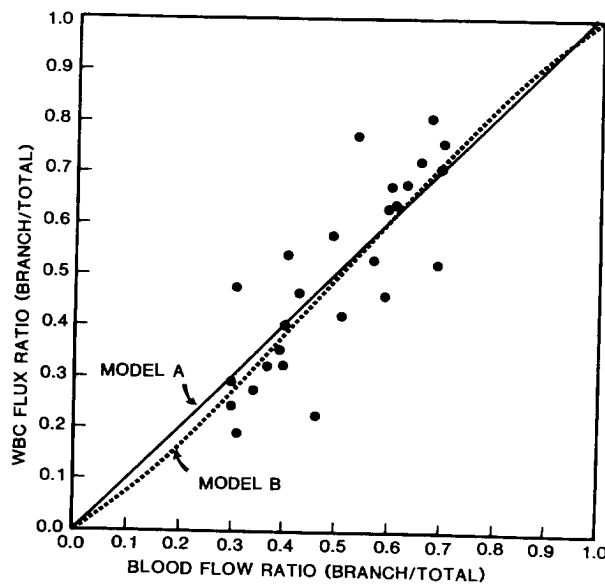


FIG. 2. Fractional flux ratio as a function of fractional flow ratio in arteriolar branches. Data (closed circles) obtained from 13 branch sites. The drawn lines represent the predicted functional relationship between F_R and Q_R according to linear (model A) and nonlinear sigmoidal (model B) models.

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TABLE 2
PARAMETER RATIOS FROM EACH PAIRED BRANCH

Branch	F_1/F_2	V_1/V_2	D_2/D_1	D_c/D_1	D_c/D_2
1	1.19	1.35	1.00	0.72	0.72
2	1.71	1.13	1.14	0.63	0.71
3	1.14	0.79	1.71	0.42	0.71
4	2.19	1.49	1.00	0.50	0.50
5	3.08	3.73	0.56	1.00	0.56
6	1.31	1.64	0.67	1.00	0.67
7	1.38	0.96	1.00	1.25	1.25
8	1.87	1.30	1.09	0.83	0.91
9	3.36	1.15	1.43	0.80	1.14
10	2.04	0.42	2.00	0.40	0.80
11	2.23	1.35	1.30	0.62	0.80
12	2.63	1.52	1.14	0.63	0.72
13	4.38	2.41	0.93	0.77	0.72

standard linear regression, $F_1/F_2 = a(V_1/V_2) + b$; linear regression with zero intercept, $F_1/F_2 = a_1(V_1/V_2)$; and nonlinear, $F_1/F_2 = a_2(V_1/V_2)^\beta$. Results show that the standard linear model yields the overall smallest residual error of the three tested and is superimposed as a solid line in Fig. 3. However, the regression coefficient is small, $r = 0.549$ and statistical significance is marginally achieved, $P = 0.052$. Parameter values and the associated standard deviations of the parameter estimates for the models are summarized as follows: $a = 0.643 \pm 0.295$, $b = 1.238 \pm 0.496$; $a_1 = 1.294 \pm 0.167$; $a_2 = 1.871 \pm 0.278$, $\beta = 0.474 \pm 0.209$.

To effectively compare the cell distribution dependence on cell velocity we

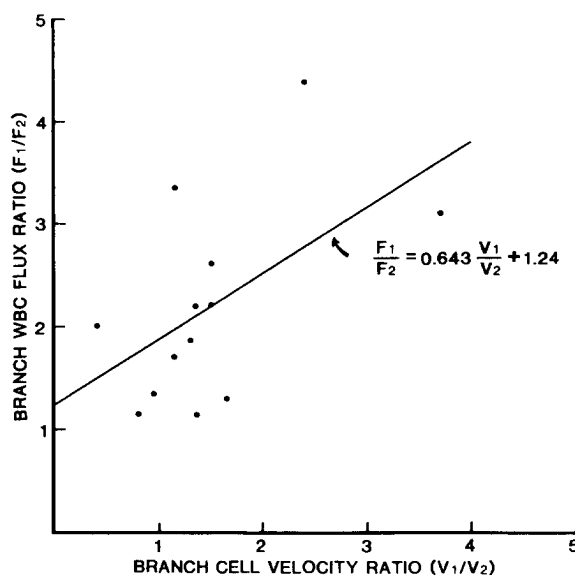


FIG. 3. Flux ratio vs velocity ratio in paired branches. Drawn line is the standard linear regression equation ($r = 0.549$, $P = 0.052$).

have obtained with available literature data, it is necessary to convert the flux ratio F_1/F_2 into a ratio of cell volume concentration in the branches, H_1/H_2 . To accomplish this, we first note that the average volume concentration, H , of n cells with an average individual cell volume, V_c , in a branch of length L , and cross-sectional area A may be given as $H = (n/L)(V_c/A)$ [Sutera *et al.* 1970]. In terms of the quantities we have measured, the average number of cells per unit branch vessel length, n/L , is given as the ratio of cell flux into that branch, $F_{1,2}$, to the velocity of cells in that branch, $V_{1,2}$. Thus the ratio of cell volume concentrations, H_1/H_2 , in the paired branches is given as $H_1/H_2 = (F_1/F_2)(V_2/V_1)(D_2/D_1)^2$. From this relationship, values for H_1/H_2 can be calculated for each branch point and plotted as a function of V_1/V_2 . The results of this procedure are plotted in Fig. 4. Statistical analyses were the same as described for the data of Fig. 3. As in that case, the standard linear regression yielded the least residual sum of squares and is superimposed on Fig. 4 as the solid line. This regression was highly significant ($P = 0.0002$) and the correlation coefficient was reasonable, $r = 0.846$. Parameter values and the associated standard deviations of the estimates for the three models as applied to this data are as follows: $a = 0.577 \pm 0.109$, $b = 0.540 \pm 0.184$; $a_1 = 0.861 \pm 0.066$; $a_2 = 1.095 \pm 0.107$, $\beta = 0.668 \pm 0.124$.

DISCUSSION

The arteriolar branches studied are one and in some cases two branching orders upstream from the capillaries. In the nomenclature of the literature they may be referred to as either terminal arterioles, precapillaries, or fourth order arterioles. In any case their diameter range was such that in no case was more

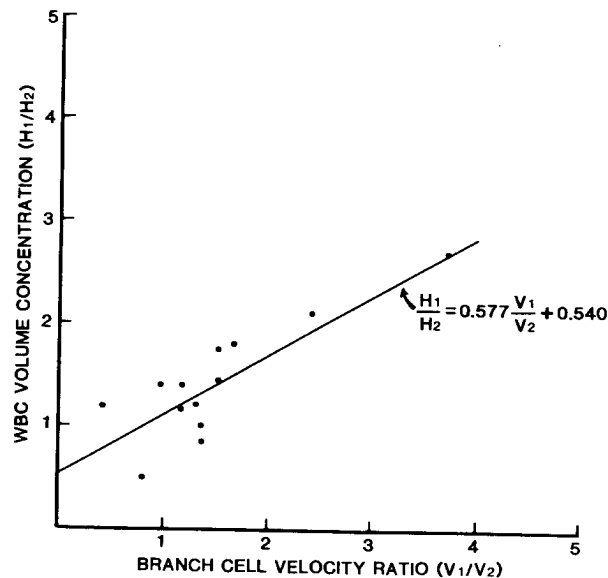


FIG. 4. Calculated WBC volume concentration vs velocity ratio in paired branches. Drawn line is the standard linear regression equation ($R = 0.846$, $P = 0.0002$).

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than one WBC observed at any cross section at the same time. Flow with respect to WBC motion could thus be characterized as single file.

The utilization of the fluorochrome, acridine orange, rendered each WBC brilliantly luminous against the dark background of the tissue under observation. This feature minimized the possibility that some WBC could pass by the observation zone without being detected either visually or utilizing the electronic sensing mechanism previously described. Further, since the acridine orange resulted in a slight staining of the endothelium the vessel outline was clearly discernable under the fluorescent microscope, permitting accurate diameter and angle measurements.

The use of the transit time of individual WBC over a known distance to estimate mean blood velocity provided a means of calculating blood flow simultaneously with the observation of cellular flux. Ordinary methods of blood velocity measurement such as the dual-slit method are not useable under conditions of low light as is necessary for the fluorescence method here employed. The accuracy with which WBC velocity reflects average red blood cell velocity in single-file motion has recently been established (Mayrovitz, 1982) and shown to be 0.95 of that which would be measured utilizing the dual-slit method.

A principle result demonstrated by this study is the predominance of blood flow as a determinant of the WBC flux, even in vessels of terminal arteriole dimensions. This blood flow domination is obtained whether one considers the absolute flux-flow relationships in all vessels studied (Fig. 1) or the distribution of cells at arteriolar branch points characterized as branch parameter ratios (Fig. 2). By examining the flux-flow ratios at such branch points for over 8000 cells a flux-flow relationship essentially indistinguishable from linearity has emerged. Factors such as diameter of individual branches or branching angles do not alter this finding over the diameter and angle range here studied. However, we reiterate the point that we cannot exclude the possibility of some degree of nonlinearity and hence preferential distribution as characterized by the flux fraction-flow fraction data. Because of the small degree of nonlinearity predicted by the nonlinear model we are not able to statistically distinguish it from a pure linear dependence. Further, since the flow ratio range encompassed by this study was about 0.3 to 0.7 the conclusions must be strictly viewed in this context. The possibility that at larger or lower flow ratios a significant nonlinearity in the flux rate may develop cannot be completely ruled out. A similar caution must be considered in the extension of these results to arteriolar branches with diameters less than those included in the present study.

Limited data in the literature is available to which comparisons may be made with regard to the branch point fractional flux-flow relationship herein obtained. For WBC distributions the data of Schmid-Schoenbein and co-workers (1980b) is particularly relevant. They found that the fractional flux-flow relationship (termed, cell distribution function) was quite nonlinear and strongly dependent on the eccentric position of the cells at the upstream entrance to the branch. This they found to be true, for white blood cells and for red blood cells within the rabbit ear microvasculature. We also have observed a similar phenomena in the hamster cheek pouch microvasculature. White blood cells in a parent vessel, having a radial position closer to the parent-branch contiguous wall, will tend

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to enter that branch, i.e., cells that are to the "left" of center in the parent tend to go into the left branch and vice versa. Thus, our observations on this point are in accord with these investigators' quantitative assessments. However, our results with regard to the extent of nonlinearity of the cell distribution function appear to be different from that presented by these workers. Their representative data (their figs. 5, 7, and 8) clearly show the strong impact of cell eccentricity on the resultant cell distribution function. For example, they point out that in some cases due to a strong bias, such as WBCs rolling on the endothelium of the left side of the parent vessel, all of the cells would enter the left branch, even though a high percentage of the bulk flow was entering the "right" branch. In our experiments few, if any, WBC were observed to be rolling in the vicinity of any branch. As a consequence this aspect of eccentricity bias and its impact on the cell distribution function in the present work is likely to be minimal. However, one should anticipate that there will exist some stochastic distribution of cellular eccentricities associated with each branch point. Related to this is the data of these authors which suggests that when the relative frequency distribution of cell eccentricity becomes more uniform, the cell distribution function becomes significantly less nonlinear. Since the WBC data per branch point presented by these workers was for at most 14 cells and as few as 5 white blood cells, it is likely that the sample size may not adequately represent an appropriate *in vivo* average characteristic. We would anticipate that if a sufficiently large number of cells per branch point were studied, then a more statistically representative uniform eccentricity distribution may have been found and hence a more linear-like cell distribution function. The data we have reported represents an average of 632 WBC per branch point. Finally, bearing on the general question of *in vivo* flow dependence of the cellular distribution at branch points is the red blood cell data of Klitzman and Johnson (1982). Their measurements of fractional flux and flow in 218 capillary branch points of the hamster cremaster microvasculature reveal a slight nonlinearity. They quote a value for the parameter b in the sigmoidal model (our model B) of 1.15 ± 0.08 SEM as compared with our value of 1.14 ± 0.18 SD. In their case they note that the nonlinearity is indeed slight but state that they are able to demonstrate that it is statistically significant.

With regard to the velocity dependence of the cell distribution at branch points the work of Yen and Fung (1978) is of direct importance. They used simulated model experiments to study the effect of model cell velocities on the cell distribution to equal diameter 180° branches. Their results indicate a linear dependence of the branch cell volume concentration ratio (H_1/H_2) on the branch cell velocity ratio (V_1/V_2) if a critical value of V_1/V_2 is not exceeded. If this critical value is exceeded, then all, or nearly all cells flow into the branch with the higher velocity. Direct comparison of our results regarding the *in vivo* WBC flux dependence on branch velocity ratios is not possible because flux, rather than branch concentrations, were measured in our study. However, by converting to branch volume concentrations as previously described we can have an indirect comparison. The result of this, as illustrated in Fig. 4, shows that our data are consistent with a linear dependence of H_1/H_2 on V_1/V_2 and in this sense our data is in accord with the model studies of Yen and Fung. However, our results have not demonstrated a critical threshold which manifests in a highly preferential distribution of cells

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to the branch with the greater velocity. It may be that this is so because the naturally occurring velocity differences we found *in vivo* were not large enough to demonstrate this phenomenon. The *in vitro* model work indicates that the critical V_1/V_2 ratio depends on the the ratio of cell diameter to tube diameter ($D_c/D_{1,2}$), and the volume concentration in the feeding vessel (H_f). In the Yen and Fung simulation, if $D_c/D_{1,2} = 0.5$, no critical threshold was demonstrated for velocity ratios over the same range we have studied. However, for $D_c/D_{1,2} = 0.67$, they found a critical threshold for V_1/V_2 of about 2.8 and 2.1 for feed hematocrit ratios of 20% and 10%, respectively. If we assume an average WBC diameter of $8 \mu\text{m}$ then our data correspond to average D_c/D_1 and D_c/D_2 ratios of 0.734 and 0.826, respectively. Thus the cell size to lumen size intrinsic to our data is close to that simulated, and a discrepancy in this parameter cannot explain the absence of a demonstrated *in vivo* threshold. The velocity ratios we encountered may have a bearing on this point. Though some of our *in vivo* V_1/V_2 data extend beyond the quoted threshold values, the bulk of our data include values less than 2.0 and this may explain why we encountered no threshold type effect. However, since the model experiments show that the velocity threshold decreases with decreasing feed vessel cell concentration we are not sure of what the critical velocity ratio for WBCs would be. In view of the normally very much lower concentration of WBCs, we might expect that a threshold for WBC, if present *in vivo*, would be less than that obtained for the 10% hematocrit case studied by Yen and Fung, who were of course primarily interested in red blood cell distribution. One final point should be noted. The two greatest velocity ratio branch points we studied clearly exceeded the *in vitro* threshold for their average $D_c/D_{1,2}$ ratio of 0.760. If an *in vivo* threshold of the type described *in vitro* were operative, major imbalances in cell distribution at these branch points would be anticipated. Such imbalances were not observed.

In conclusion, in the present report we have not attempted to characterize the dynamics of individual leukocytes. Therefore such factors as the radial position of the cell as it approaches the bifurcation, the precise shape of the bifurcation geometry, the specific size and physical properties of each cell, and the effect of cell entry in one branch on the subsequent events in the other branch have not been explicitly dealt with. Rather, it should be emphasized that the WBC flux-flow relationship established by the present work is applicable as an average characterization. Thus the interpretation should be that the average number of white blood cells delivered to the distal capillary bed through a given branch pathway is principally dependent on the average blood flow in that branch. Clearly, an array of factors including those listed above have some effect on this overall *in vivo* property. However, our observations and analysis indicate that the summated effects yield the average flux-flow dependency as described.

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