STICKING SHEAR FORCE IN VENULES

By Geert W. Schmid-Schoenbein, Yuan-Cheng Fung, and Benjamin W. Zweifach

ABSTRACT
To determine the shear force acting on a white blood cell sticking to the endothelium of a blood vessel, the flow field about a single white blood cell in a venule was determined by high-speed motion picture photomicrography. The force acting on the white blood cell was then calculated according to the principles of fluid mechanics. In this paper, the calculation was made using an experimentally determined dimensionless shear force coefficient obtained from a kinematically and dynamically similar model. The large physical model of the hemodynamic system could be easily instrumented, and the shear force acting on the model cell and the flow field around it were measured. The data were then used to calculate a shear force coefficient. On the basis of dynamic similarity, this shear force coefficient was applied to the white blood cell in the venule. The shear force coefficient was strongly influenced by the hematocrit, so in vivo hematocrits were measured from electron micrographs. It was found that in the venules of the rabbit omentum a white blood cell sticking to the endothelial wall was subjected to a shear force in the range of $4 \times 10^{-5}$ dynes to $234 \times 10^{-5}$ dynes; the exact value depended on the size and motion of the white blood cell, the size of the blood vessel, the velocity of the blood flow, and the local hematocrit, which varied between 20% and 40% in venules of about $40 \mu m$ in diameter. The contact area between the white blood cell and the endothelial cell was estimated, and the shear stress was found to range between 50 dyne/cm² and 1060 dyne/cm². The normal stress of interaction between the white blood cell and the endothelium had a maximum value that was of the same order of magnitude as the shear stress. The accumulated relative error of the experimental procedure was about 49%. The instantaneous shear force was a random function of time because of random fluctuations of the hematocrit.

KEY WORDS
physical modeling  mechanical stress  circulating white blood cells  rolling leukocytes  inflammation  large-scale model experiments  hematocrit  omentum  rabbit
to the genesis of atherosclerosis. Fry created high shear stress in the aorta by inserting into the vessel a cylindrical plug that contained a groove on the surface. The blood was forced to flow into the groove, and its velocity was increased by the reduction in cross-sectional area. In normal blood vessels, the shear rate is much smaller than those created by Fry. We wondered, however, whether Fry's critical yield stress is comparable to the stress of interaction between white blood cells and endothelial cells. If these stresses are comparable (they were found to be so), then, since the vascular endothelium is constantly pelted by the white blood cells, the interacting shear stress may be relevant to atherogenesis.

The adherence of white blood cells to the endothelium of the blood vessels is a manifestation of inflammation on the microcirculatory level. Florey (3) has reported the sticking of white blood cells on inflamed venular vessel walls. Allison et al. (4) have reported on the rolling of white blood cells on the arteriolar wall in instances in which the velocity of the bloodstream is markedly reduced. Fry (2) has observed white blood cells attached to the endothelium of thoracic arteries. Atherton and Born (5, 6) have quantified the interaction between leukocytes and blood vessel walls by measuring the number and the velocity of granulocytes rolling on a segment of the wall. They considered the effect of chemical intervention on "adhesiveness" and presented an estimation of the shear force between the granulocytes and the endothelium, but they did not give an account of how this interacting force can be computed rigorously.

It is the purpose of this article to present a method of determining the shear force acting on a leukocyte sticking to the wall of a venule at various rates of blood flow. The in vivo flow field about the leukocyte was obtained by high-speed photography. The mathematical problem of calculating the force of interaction was replaced by testing a physical model. A kinematically and dynamically similar model of the venule-endothelial system was made. The model had a convenient size so that the flow and the forces could be measured accurately. By the principle of dynamic similarity, the dimensionless shear force coefficient should be the same for the blood vessel and the model. Thus, the experimental shear force coefficient could be used to calculate the force acting on the leukocyte.

**Analysis**

Two flow systems are said to be kinematically similar if their geometric shapes are similar; their linear dimensions can be different. Two systems

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>$d_L$</td>
<td>Diameter of the leukocyte considered as a sphere</td>
</tr>
<tr>
<td>$d_v$</td>
<td>Diameter of the blood vessel</td>
</tr>
<tr>
<td>$V_m$</td>
<td>Maximum velocity of undisturbed flow in the blood vessel</td>
</tr>
<tr>
<td>$V_c$</td>
<td>Linear velocity of the centroid of the white blood cell</td>
</tr>
<tr>
<td>$\mu$</td>
<td>Coefficient of viscosity for plasma</td>
</tr>
<tr>
<td>$\rho$</td>
<td>Density of plasma ($\rho$ is that of the silicone oil)</td>
</tr>
<tr>
<td>$H$</td>
<td>Hematocrit of the blood, i.e., the volume fraction of the cellular content in the blood</td>
</tr>
</tbody>
</table>

All symbols with an overbar refer to the model. For example, $\bar{\mu}$ signifies the viscosity of plasma and $\bar{\mu}$ denotes the viscosity of the silicone oil in the model experiment. They are said to be dynamically similar if the systems of differential equations describing their motion, written in dimensionless form, are the same. If two systems are both kinematically and dynamically similar, then the dimensionless variables (stresses, velocities, displacements, etc.) will have exactly the same value at corresponding points in the two systems. This is the basic principle of similarity (see, for example, Langhaar, 7).

In the blood flow problem, the motion of the plasma is governed by the Navier-Stokes equation, because plasma is known to be Newtonian. The non-Newtonian properties of whole blood are due to the suspended cellular bodies, which are simulated by gelatin pellets in our experiment. Written in dimensionless form, the Navier-Stokes equation contains only one dimensionless parameter, namely, the Reynolds number (8). Hence, two geometrically similar flows can be made dynamically similar simply by making their Reynolds numbers equal.

A dimensional analysis for modeling the motion of a red blood cell in a capillary blood vessel has been presented by Lee and Fung (9). A similar analysis can be made for the problem of a white blood cell in a venule. Let the principal variables describing the geometry and motion of our system be those listed in Table 1. In this table, $H$ is dimensionless. From the remaining six variables, three independent dimensionless variables can be formed. The following is a convenient set:

$$d_L/d_v, V_c/V_m, V_m d_L p/\mu.$$

All other dimensionless parameters are functions of these basic variables. For example, if we are interested in the resultant shear force, $S$ (dynes), imparted to the white blood cell by the blood flow, we note that the ratio $S/(V_mkd_L)$ is dimensionless;
we can express this ratio as

\[
\frac{S}{V_{m}d_c} = f\left(\frac{d_c}{d_v}, \frac{V_c}{V_m}, \frac{V_m \mu}{\rho}, H\right),
\]

where \( f(\ ) \) denotes a functional relationship that can be determined either by solving the differential equations or by using a model experiment. We notice that the parameter \( V_m \mu / \rho \) is a Reynolds number \( (R_e) \). The other parameter \( S/(V_m \mu d_c) \) will be called the shear coefficient and denoted by \( C_s \); thus,

\[
C_s = \frac{S}{V_{m}d_c}, \quad (1)
\]

\[
R_e = \frac{\rho V_m d_c}{\mu}, \quad (2)
\]

\[
C_s = f(R_e, d_c/d_v, V_c/V_m, H). \quad (3)
\]

If we use a bar over a variable to denote quantities belonging to a geometrically similar model, then

\[
\bar{C}_s = f(\bar{R}_e, \bar{d}_c/\bar{d}_v, \bar{V}_c/\bar{V}_m, \bar{H}). \quad (4)
\]

The basic principle of the present paper is simply to determine Eq. 4 by model experiments, then to identify \( \bar{C}_s \) with \( C_s \) in Eq. 3, and to use Eq. 1 to compute the shear force \( S \):

\[
S = C_s V_m d_c. \quad (5)
\]

In the preceding analysis, parameters that define the shape and the elasticity of the white blood cells, the red blood cells, and the other cellular bodies were omitted. These parameters were simulated as best we could, but imperfectly, as will be discussed later.

It turns out that the shear coefficient \( C_s \) is strongly dependent on the hematocrit. Since the hematocrit is stochastic in vivo, the shear force must fluctuate as time passes. The fluctuation is quasi-static, because the Reynolds number of flow is very small. In the following analysis, \( H, C_s, S, \) etc. are presented in terms of their mean values and their deviations from the mean.

Inertial force due to fluid acceleration is negligible. The motion of the white blood cell is comparatively slow. Furthermore, because its body is so small and because it is suspended in a fluid of almost equal density, the body force is insignificant. The resultant force in the axial direction can also be neglected for the following reason. The pressure drop in a venule with an average length of 2000 \( \mu \)m is at most on the order of 5 mm Hg. A sphere with a radius of 5 \( \mu \)m situated in such a pressure field will be subjected to a resultant force on the order of \( 3 \times 10^{-8} \) dynes. The resultant pressure force in the axial direction is on the order of \( 10^{-7} \) dynes. As will be seen later, the resultant of the shear stresses acting on the white blood cell is on the order of \( 10^{-4} \) dynes. Hence, in the axial direction, the resultant of the
pressure forces is small in comparison with the resultant of the shear stresses. Thus, the total drag force is approximately equal to the resultant of the shear stress acting on the white blood cell.

Since the inertial force is negligible, the balance of forces in the axial direction demands that the resultant shear force acting on the white blood cell by the endothelium be equal to $S$. The endothelium is subjected to the same shear $S$.

The resultant of the normal stresses due to curvature of the streamlines around the white blood cell, caused by the non-Newtonian feature of the suspension, acts mainly in the radial direction, which is not of concern in the present paper. But this resultant radial force may be important in explaining why the white blood cells are pressed so hard onto the endothelium when they do adhere, as evidenced by the large values of $L_p$ (not negligible compared with the cell diameter $d_c$ in Tables 3 and 4).

The normal stress $\sigma$ acting on the interface between the endothelium and the white blood cell must balance the normal component of the pressure and shear forces acting on the cell. Since the surface traction acting on the white blood cell has a resultant torque $SL$ which does not vanish, where $L$ represents the moment arm as shown in Figure 1, the normal stress $\sigma$ on the interface cannot be uniform and must have a resultant moment equal to $SL$.

The preceding analysis is applicable both when the white blood cell rolls at a constant speed and when it remains stationary. If the torque $SL$ exceeds the torque of the normal stresses acting on the interface, then the rolling of the white blood cell will be accelerated. The normal stress of interaction between the endothelium and a stationary white blood cell will be maximum when the cell is on the verge of rolling down the wall. The shear stress acting on the surface of the white blood cell will be affected by rolling of the cell. Atherton and Born (6) have shown that the average rolling velocity of the white blood cell is only about 4% of the mean blood flow velocity in the venule. Our measurements (see Table 4) confirm this finding. With such a small rolling velocity, the flow pattern around the rolling white blood cell should be almost the same as that around a stationary cell. However, with rolling, there might develop a lubrication layer between the white blood cell and the endothelium similar to that considered by Light hill (10) in his theory of motion of red blood cells in the capillary blood vessels. In that case, the drag on the cell will be less than that on a stationary cell. Note, however, that the area of contact between a white blood cell and the endothelium is not significantly reduced by rolling (see Tables 3 and 4, compare $L_p$ with $d_c$), which shows that the white blood cell is not lifted off the endothelium. This fact suggests that the lubrication layer effect, if it exists, is not very significant.

If the interaction between the white blood cell and the endothelium is such that the maximum shear stress on the interface cannot sustain the resultant shear force $S$, then the white blood cell will be accelerated downstream by a sliding motion. Whenever the white blood cell is not accelerated, the resultant shear on the interface must balance the shear $S$. It is, therefore, of interest to determine whether the impending motion of the white blood cell, when it is on the verge of being swept away by the blood flow, is rolling or sliding. If it is rolling, then we can compute the maximum normal stress of interaction. If it is sliding, then we can obtain the maximum shear stress.

Methods

Animal Preparation and Photography.—A high-speed camera (Hycam) was mounted over a Leitz intravital microscope, using transmitted light from a 900-w xenon source, a 20x, 32x, or 40x objective, and a 10x eyepiece. Photographs were taken at a speed of 1800–2800 frames/sec on 16-mm black-and-white film (92 ASA). Photographs were taken at a speed of 1800–2800 frames/sec on 16-mm black-and-white film (92 ASA). White New Zealand rabbits (1–1.3 kg) were anesthetized with 50 mg/kg of the barbiturate Inactin, supplemented with 5 mg/kg at 20-minute intervals. The omentum was exteriorized through a small abdominal incision; precautions were taken to avoid damage to this structure. No stimulation was necessary to observe white blood cell adhesion in the venules. The omentum was irrigated with Ringer's solution at pH 7.2, and the rabbit as well as the stage with the omentum was kept at 37°C. We found that by reducing the hematocrit the contrast between the adherent leukocyte and the blood-stream could be significantly improved. Therefore, in selected experiments, the systemic hematocrit was reduced to about 25% from a control level of about 40% by withdrawing blood and replacing it with an equivalent volume of 5% serum albumin in isotonic saline. The central aortic mean pressure was unaffected by such a procedure and remained at 80–90 mm Hg.

The microscope was focused as sharply as possible on a middle plane of the blood vessel as shown in Figure 2. The diameter of the venules ranged between 20 μm and 55 μm. To deal with a fully developed, undisturbed velocity field, we selected long straight vessels with a single adhering white blood cell. A calibrated time and a length scale then made it possible to measure from the film the maximum velocity, $V_w$, the diameter of the cell, $d_c$, and the diameter of the blood vessel, $d$. The accuracy of these measurements will be discussed later.

Hematocrit.—In vivo hematocrits in venular vessels were measured according to a principle which was applied by Weibel (11) to find the volume ratio of tissue components in the lung. We defined the hematocrit $H$ in a venule as the volume of red blood cells, white blood...
A 23-μm postcapillary blood vessel with a leukocyte (WBC) adhering to the endothelium. The time elapsed between the successive frames shown in the figure is 3 msec. The small arrows in frame 1 point to endothelial cells. The black bar on the lower boundary of the vessel marks a recognizable characteristic point on the wall. A red blood cell (ER) moved from left to right in successive frames of the motion picture.

cells, and platelets in a vessel segment divided by the enclosed blood volume. Weibel points out that under the condition of randomly distributed cells one can make a section in any arbitrary plane, measure the fraction, \( H_s \), of the area covered by the transected structure—in this case the blood cells—and state that

\[
H = H_s, \quad (6)
\]

irrespective of the shape of the vessel whose volume is being considered. In the venules (25-50 μm), the size of red blood cells is not negligible, and the distribution varies instantaneously, apparently never attaining a completely random state. In those instances in which it is possible to increase the cross-sectional area on which the measurements of \( H_s \) will be made, the approximation to \( H \) is greatly improved; in fact, by taking a greater number of serial sections, the calculated values can approach the hematocrit to any desired accuracy. The experimental error involved when \( H_s \) was obtained from a central axial cut of a circular cylinder was tested as follows.

Black-stained circular gelatin pellets (diameter 1.18 cm, thickness 0.3 cm) made from Chinese agar-agar (1 g/100 ml distilled water), as extensively used by Yen and Fung (12), were suspended in a solution of cold gelatin (6 g/150 ml H₂O). The suspension was then refrigerated. Prior to solidification, the cooled mixture was poured into a plastic circular cylindrical tube (2.5 cm, i.d., 3.8 cm, o.d.), and slices were then made of the gel column through the axis. The surface ratio of the pellets, \( H_s \), was measured with a planimeter from a photograph of such cross sections. Subsequently, the two half-cylinders were glued together with cold gelatin, a new cut was made at a right angle to the first, and the measurements were repeated. The experimental results are:

**Tube 1**  
Area = 60 x pellet cross section (1.09 cm²).

Cut 1 \[ \frac{|H - H_s|}{H} = 0.07, \]

Cut 2 \[ \frac{|H - H_s|}{H} = 0.013. \]

**Tube 2**  
Area = 98 x pellet cross section.

Cut 1 \[ \frac{|H - H_s|}{H} = 0.065, \]

Cut 2 \[ \frac{|H - H_s|}{H} = 0.05. \]

As the area is reduced, the positive correlation between \( H \) and \( H_s \) is jeopardized.

The in vivo hematocrits were measured with planimetry from electron micrographs¹ (see ref. 13 and

¹ The authors want to thank Dr. Johannes Rhodin for his cooperation in providing us with these beautifully prepared electron micrographs.
bubbles were introduced into the silicone oil simply by pouring the oil into the container. A camera lens with a short depth of field (3 mm) was focused on the plane of symmetry of the sphere and the tube. The maximum velocity, $V_M$, as measured from 16-mm film recordings, was found to correspond to those in the animal experiment. When the maximum velocity of the air bubbles was compared with the velocity computed on the basis of Poiseuille flow at the known flow rate, the difference varied between 3% and 12% of the calculated value.

For an additional check, the plasma was also modeled by silicone oils with viscosities of 100 and 125 poises. In this case, the force measurement was modified as follows. The sphere was attached to a surgical silk thread which was passed through a 1-mm boring in the Plexiglas wall at an angle of 60° with reference to the tube wall and then suspended on a force transducer. The cell was touching the wall at the boring. The leakage of fluid during the experiment was negligible because of the small size of the opening containing the thread and the high viscosity of the silicone oil. Friction of the thread in this boring caused a small transient disturbance which lasted less than 0.5 seconds. The steady-state shear force was recorded.

We also tested a different method of velocity measurement, which used a television camera in conjunction with dual-window video-densitometry and cross-correlation technique (14, 15). The camera was focused on the same plane of symmetry; the windows were positioned at a known distance apart on the axis of the tube. The velocity of flow could be calculated from the measured delay time. A check of both methods of velocity and force measurements with a 47-poise silicone oil on one sphere yielded the same relationship between the shear force and $V_M$.

The same arrangement was used to measure the shear force on a white blood cell model ($d_c = 0.63$ cm) in a particulate flow simulating whole blood. In this case, the red blood cells were modeled by gelatin pellets (shear modulus $7.7 \times 10^3$ dynes/cm²). The pellets had a rectangular form $0.7 \times 0.7 \times 0.2$ cm. The pellet size was much smaller than the tube diameter. Differences in velocity profile in the tube caused by small changes in pellet shape are expected to be small. The effect of pellet shape on the shear force acting on the white blood cell model is not known but is believed to be a minor factor. The rigidity of the pellets (compared with that of red blood cells) is believed to have a greater effect, but the effect of red blood cell deformability on flow relationships in such tubes has not been quantified. The pellets and the silicone fluid (100 poise) were mixed carefully to form homogeneous suspensions at three different hematocrits. The velocity of flow was controlled by a vacuum tank connected to the lower end of the tube using a 1-m long hose filled with silicone oil. At the higher hematocrit, direct observation of the midstream velocity was not possible; hence, we determined the volume flow by measuring the displacement velocity of the free surface of the liquid in the container multiplied by its cross-sectional area. The center-line velocity of flow was then calculated from Poiseuille's formula. The shear force $S$ was plotted as a function of time on a paper chart recorder. This arrangement is the same as that used by Yen and Fung (12).

The diameter of the model cell, $d_c$, was measured directly, the viscosity, $\mu_c$, was checked with a tube
Results

At zero hematocrit the model experiment was performed with spheres of several diameters. Figure 4 shows a linear force-velocity relationship for three cells at a fluid viscosity of 125 poise. It indicates also that the drag force is a function of the cell diameter. A representative protocol in which the shear force coefficient $C_s$ was calculated from Eq. 1 as a function of the Reynolds number is shown in Figure 5. Other experiments were done in which the sphere diameters, $d_c$, were 0.4, 0.55, and 0.63 cm and the fluid viscosities 47, 100, and 125 poises. Although the range of Reynolds numbers which are applicable to the animal experiments is only around 0.01, these measurements were made up to a value of 0.1 to obtain a general trend of $C_s$ as a function of Reynolds number. The experimental results show that $C_s$ increases with increasing Reynolds number and increasing $d_c/d_t$. Hand-fitted lines to data typically represented in Figure 5 can be expressed by the following:

$$\frac{d_c}{d_t} = 0.17 \quad C_s = 6.2 + 80Re,$$

$$\frac{d_c}{d_t} = 0.24 \quad C_s = 8 + 80Re,$$

$$\frac{d_c}{d_t} = 0.28 \quad C_s = 8.8 + 80Re.$$

Table 2 lists a sample of venular hematocrits in the fascia. In the right column, the term serial cuts refers to the fact that the hematocrit was obtained as the average value of the surface ratios, $H_s$, at several depths in each blood vessel segment. The values of $H_s$ do not vary appreciably, supporting the hypothesis that the distribution of red blood cells in vessels of this size is not significantly different from a random distribution. The other hematocrits are from vessels pictured by a single cut. We conclude from Table 2 that in venules with a diameter of 30-40 $\mu m$ average hematocrits can vary 20-40%. The instantaneous local hematocrit in the proximity of a sticking white blood cell would be expected to vary over this range of limits.

Figure 6 shows the shear force $S$ as a function of time for a fluid with a hematocrit of 30% in the container and 27% in the tube. The three curves correspond to three different maximum velocities; they clearly indicate that the mean shear force, as well as the maximum deviations, increase with increasing velocity of flow. At higher velocities the pellets stream faster past the model cell, resulting in sharper peaks of the shear force $S$. This feature was observed at all hematocrits. The shear force $S$ on one model cell ($d_c = 0.63$ cm) was calculated as a function of the average center-line velocity $V_M$ for a fluid with a viscosity of 100 poise containing pellets at three different hematocrits, 20%, 30%, and 40%. Figure 7 shows the data for a hematocrit of 30%. The solid circles indicate the mean values averaged over a period of about 25 seconds, and the vertical bars indicate maximum deviations from
TABLE 2
Venular Hematocrits

<table>
<thead>
<tr>
<th>d_v × 10^{-4} cm</th>
<th>H = H_s (%)</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>26</td>
<td>Single cut</td>
</tr>
<tr>
<td>30</td>
<td>22</td>
<td>Serial cuts (single venular segment)</td>
</tr>
<tr>
<td>30</td>
<td>19</td>
<td>Serial cuts</td>
</tr>
<tr>
<td>30</td>
<td>29</td>
<td>Serial cuts</td>
</tr>
<tr>
<td>31</td>
<td>28</td>
<td>Single cuts</td>
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<tr>
<td>32</td>
<td>37</td>
<td>Serial cuts</td>
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<td>73</td>
<td>33</td>
<td>Single cut</td>
</tr>
<tr>
<td>120</td>
<td>39</td>
<td>Serial cuts</td>
</tr>
<tr>
<td>120</td>
<td>39</td>
<td>Serial cuts</td>
</tr>
</tbody>
</table>

d_v = venular diameter, H = hematocrit, and H_s = average value of the surface area ratios.

The results of the animal experiment are exhibited in Tables 3 and 4. In columns 4, 6, and 8 of each table some calculated results based on the model experiments at zero hematocrit are shown. The viscosity of plasma is taken to be \( \mu = 1.2 \times 10^{-2} \) g/cm sec\(^{-1}\) (16). The values of \( \bar{C}_0 \) were determined for the corresponding values of \( Re \) and \( d_v/d_s \) by interpolating linearly from the experimental values. The computed shear force \( S \) is shown in column 6 of Tables 3 and 4. Table 3 refers to those white blood cells that were stationary on the endothelium, and Table 4 refers to (1) white blood cells about 10^{-2} seconds before they were carried away by the bloodstream from a stationary position (group A) and (2) cells rolling at constant speeds (group B). In this last case, the velocity of translation of the white blood cells never exceeded 0.007 cm/sec, a velocity much slower than \( V_M \). Our results show that the resultant shear force, \( S \), acting on a white blood cell rolling on the endothelial wall lies in the range of 4 to 45 \( \times 10^{-6} \) dynes if the hematocrit is zero. By taking the mean values from Figure 7 and similar plots, the shear force at finite hematocrits is estimated to be:

\[
\begin{align*}
8-90 \times 10^{-6} \text{ dynes at } H = 20\%, \quad & (7a) \\
16-180 \times 10^{-6} \text{ dynes at } H = 30\%, \quad & (7b) \\
21-234 \times 10^{-6} \text{ dynes at } H = 40\%. \quad & (7c)
\end{align*}
\]

Instantaneous maximum values increase to higher values. The precise value of the shear force depends on the size of the white blood cell, the size of the blood vessel, the velocity of blood flow, and the instantaneous hematocrit.

An estimation of the magnitude of shear stress cannot be made with any certainty, because it is difficult to determine the area of contact between the white blood cell and the endothelium, since the light at that point is significantly diffracted. To have some idea of the order of magnitude, however, we estimated the length \( L \) of the projected contact area, as shown in column 7 of Tables 3 and 4 for a number of white blood cells. If we assume a plane circular area of contact, with \( L_p \) as the diameter, we obtain the shear stress by dividing \( S \) by the area \( \pi [L_p/2]^2 \). The result is given in the last column of Tables 3 and 4. The shear stress varies approximately between 50 dynes/cm\(^2\) and 200 dynes/cm\(^2\) at zero hematocrit; hence, it assumes in the mean 100-400 dynes/cm\(^2\) at \( H = 20\% \).
200–800 dynes/cm² at $H = 30\%$, and 265–1060 dynes/cm² at $H = 40\%$. Instantaneous shear stress can be higher.

It would be interesting to estimate the maximum normal stress of interaction between the white blood cell and the endothelium. For this purpose let us assume that the normal stress is a linear function of $x$, as shown in the lower sketch.

### Table 3

Experimental Results for Those White Blood Cells that Adhered to the Blood Vessel Wall without Motion and Calculated Values for the Reynolds Number, Shear Force Coefficient at Zero Hematocrit, Shear Force, and Shear Stress

<table>
<thead>
<tr>
<th>$V_u$ (cm/sec)</th>
<th>$d_c \times 10^{-4}$ (cm)</th>
<th>$d_c/d_t$</th>
<th>$R_s$</th>
<th>$C_s$</th>
<th>$S \times 10^{-6}$ (dynes)</th>
<th>$L_p$ ($\mu$m)</th>
<th>$\tau = S/A$ (dynes/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.18</td>
<td>8</td>
<td>0.22</td>
<td>0.013</td>
<td>9.0</td>
<td>15</td>
<td>6</td>
<td>55</td>
</tr>
<tr>
<td>0.18</td>
<td>6.5</td>
<td>0.17</td>
<td>0.011</td>
<td>7.0</td>
<td>10</td>
<td>5</td>
<td>50</td>
</tr>
<tr>
<td>0.18</td>
<td>9</td>
<td>0.24</td>
<td>0.015</td>
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$V_u$ is the maximum speed of flow in the venule, $d_c$ is the diameter of the white blood cell, $d_t$ is the diameter of the venule, $R_s$ is the Reynolds number based on plasma viscosity, $C_s$ is the shear force coefficient assuming hematocrit is zero and based on model experiments, $S$ is the resultant shear force acting on the white blood cell (calculated based on plasma viscosity, $L_p$ is the diameter (2a in Fig. 1) of the contact area between the white blood cell and the endothelium, and $\tau = S/A$ is the shear stress between the white blood cell and the endothelium if the hematocrit is zero. $S$ and $\tau$ are larger if the hematocrit is not zero (see Fig. 7 and text).

### Table 4

Experimental Results for Those White Blood Cells that Adhered Statically to the Venule Wall and Then Started to Move and Were Carried away by the Blood Flow (Group A) and Those that Rolled along the Endothelial Wall (Group B)

<table>
<thead>
<tr>
<th>Group</th>
<th>$V_u$ (cm/sec)</th>
<th>$d_c \times 10^{-4}$ (cm)</th>
<th>$d_c/d_t$</th>
<th>$R_s$</th>
<th>$C_s$</th>
<th>$S \times 10^{-4}$ (dynes)</th>
<th>$L_p$ ($\mu$m)</th>
<th>$\tau = S/A$ (dynes/cm²)</th>
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Abbreviations are the same as they are in Table 3. Data in the maximum velocity of flow in the center of the venule for group A were measured from frames of motion pictures taken about 10^{-2} seconds before such movements. $S$ and $\tau$ were calculated for zero hematocrit; if the hematocrit is not zero, $S$ and $\tau$ are larger (see Fig. 7 and text). The data for group B correspond to those for group A; the axial velocity of the centroid of the white blood cells in group B was less than 70 μm/sec.
of Figure 1. Let the area of contact be a circle of radius \( a \), and let the maximum normal stress at the outer edge be \( \sigma_0 \). Then the total moment is

\[
4 \int_{0}^{\pi/2} \sigma_0 a^3 \sin^2 \theta \cos^2 \theta \, d\theta = \frac{\sigma_0 a^3}{4} \pi,
\]

which must be equal to the torque \( SL \) as discussed before in the section on analysis. Hence,

\[
\sigma_0 = \frac{8SL}{\pi a^3},
\]

For the assumed white blood cell considered previously, with \( S = 10^{-5} \) dynes, \( a = 2.5 \mu m \), and \( L = 3.5 \mu m \), we obtain

\[
\sigma_0 \approx 285 \text{ dynes/cm}^2.
\]

The corresponding shear stress, assuming a constant stress distribution, is 50 dynes/cm². These calculations indicate that the normal stress of interaction is probably of the same order of magnitude as the shear stress, but it may be several times larger.

The calculation of the normal stress is based on the simplifying assumption that the normal stress varies linearly with the distance from the centroid of the contact area. This assumption may very well be incorrect. However, even if we assume a uniform tension for \( x < 0 \) and a uniform compression for \( x > 0 \), we will still obtain a stress \( \sigma = SL/(4a^3) \), which, in the numerical example given previously, amounts to \( \sigma_0 = 168 \text{ dynes/cm}^2 \).

Our observations concerning the question of sliding or rotating movement of the white blood cells on the endothelium are based on direct microscopy and black-and-white high-speed motion picture films. When the white blood cell travels over longer distances, its rotation can be recognized by the movement of its outer contour. White blood cell rotation was also noted when the cell first came into contact with the vessel wall. On the other hand, we also observed white blood cells which were deformed by the bloodstream, had no rotation of their contour, and slid over distances of approximately their own diameter before they stopped, started to roll, or were carried away by the bloodstream. The velocity of this sliding is significantly lower than \( V_M \), similar to that of the rotating movement. Because of its transient character, the sliding movement is not included in Table 4. Finally, we should mention that rolling without sliding was found for 40 leukocytes which were studied carefully in the rabbit omentum. In such cases, the maximum normal stress will probably be smaller than that estimated by Eq. 9.

**Discussion**

Inasmuch as blood plasma is known to be a Newtonian fluid, it is permissible to simulate it with silicone oil, thus making it possible to establish accurately the maximum error of the shear force at zero hematocrit and thereby to evaluate independently the additional error involved when blood cells are simulated as well.

A graphical method showed that both the velocity and the corresponding shear force acting on the model had a maximum relative error of 14% (from Fig. 4 for \( d_c = 0.4 \text{ cm} \)). Repeated measurements of the viscosity and the cell diameter indicated an error of less than 1% and 2%, respectively; hence, the maximum error of the model test adds up to
31%. The diameter of a white blood cell taken from the high-speed motion pictures contained an 8% relative error (determined by triplicate measurements of a number of single cells at all three magnifications applied and calculation of the maximum deviation from a mean diameter). The probable error in the velocity as determined by the same principle was 10%, primarily due to light diffraction and red cell rotation. When these values are added together, we find a 49% maximum relative error of the shear force at constant viscosity on a white blood cell.

The geometric modeling of the white blood cell and the blood vessel were idealized. The walls of living real venules are not smooth cylinders, and the actual white blood cells are not spheres. Although white blood cells are deformable particles, in the venule they frequently appear to be spherical. Within the limits of resolution and the twodimensionality of the high-speed photographs, the present study includes only data for those white blood cells that are not distorted. Therefore, only spheres were used in our modeling. Some experiments were done with teardrop-shaped bodies \( \overline{d_c} = 0.45 \text{ cm} \) at zero hematocrit; the results showed that their shear coefficients \( C_s \) fell within the scatter band of Figure 5.

The shear force in Tables 3 and 4 was calculated from Eq. 5 with values of \( C_s \) corresponding to zero hematocrit. The actual hematocrits in the animal experiments during the inflammatory process with sticking white blood cells varied with time. We therefore elected to indicate the range of the shear force (Eq. 7) on the basis of the model experiment for mean venular hematocrits established by direct measurements. In the latter study, the red blood cells were simulated by gelatin pellets. These pellets are less deformable than red blood cells; thus, the apparent viscosity of the simulated blood is probably higher than that of the real blood. Hence, the shear force must lie within the bounds of the values given in Tables 3 and 4 for zero hematocrit and those given by Eq. 7 with \( C_s \) given by pellets experiments. Unfortunately, we do not know how to make liquid-filled artificial cells as flexible as real red blood cells. The real red blood cell membrane can be stretched several hundred percent with virtually no change in area. No rubber can do so. Neither do we know how to simulate the electrical properties (particularly the double layer) of the red cell membrane. Hence, future improvements must be awaited. However, the simulation presented by Yen and Fung (12), using gelatin pellets in silicone oil in a lung model, compares favorably with in vivo experimental results as far as the effect of hematocrit on viscosity is concerned.

The approximation of venular hematocrits made by surface ratios, \( H_s \), includes the uncertainty that electron micrographs may not reveal the spatial distribution of blood cells. It is thus not possible from case to case to estimate the uncertainty factor for \( H \). The serial cuts are a better approximation and indicate that the maximum error of \( H_s \) is less than 9% of the mean value for all of the surface ratios in a particular vessel. Shrinkage in the preparation has been reported by Rhodin (13) to be negligible so that no correction was made for this contingency.

Five readily distinguishable types of white blood cells are reported to participate in the inflammatory reaction in mammalian tissues with the granulocytes being the most numerous, 85%. These types are: (1) granulocytes (diameter \( \approx 10-15 \mu \text{m} \)), (2) eosinophils (10-13 \( \mu \text{m} \)), (3) basophils (7-10 \( \mu \text{m} \)), (4) monocytes (10-12 \( \mu \text{m} \)), and (5) lymphocytes (10 \( \mu \text{m} \)). All of these cells have a tendency to adhere to vascular endothelium (3, 17). The inflammatory reaction is accompanied by vasodilation and a marked increase in blood flow. Since our experiments show that the interacting force depends on the flow velocity, it is clear that the blood flow velocity is an important determinant of the type and size of white blood cells which will adhere to the endothelium, and, subsequently, extravasate into the neighboring tissue.

Shear rates and shear stresses on the endothelium have been measured previously in large thoracic blood vessels of different species. Ling et al. (18) calculated the shear stress from measured velocity profiles in the aorta of the dog and the pig. They obtained an average value between 80 and 160 dynes/cm\(^2\) in normal flow. Fry (1, 2) created high-shear flow by means of a plug and determined critical yield stress. As mentioned before, Fry's yield stress is of the same order of magnitude as the interacting shear force between white blood cells and vascular endothelium. These facts suggest that the shear stress in large arteries and small veins is of the same order of magnitude under in vivo flow conditions.

It is well known that the number of white blood cells sticking to the endothelium varies with the degree of injury to the tissue and the time after injury, but it is not generally appreciated how small a disturbance will qualify as "injury." The white blood cells examined and reported on in this paper were found in the rabbit omentum and mesentery after these tissues had been gently...
taken out of the animal and laid out under the microscope. This gentle handling by an experienced investigator was sufficient to cause accumulation, sticking, and rolling of white blood cells on the endothelium of the venule. The reaction subsides in time. Atherton and Born (5) have reported on the course of events.

We can measure the shear rates and shear stress in venules from our high-speed motion pictures. In the rabbit omentum, we estimated that the high value of shear stress was 50 dynes/cm² on the basis of the smallest clearance between the endothelium and the nearest red blood cell, the centroidal velocity of the red blood cell, and the plasma viscosity. Estimates of this kind, however, are affected by the diffraction of light by the blood vessel.

Finally, a remark about the choice of the dimensionless coefficient \( C_n \) should be made. In aerodynamics text books, the drag coefficient is defined by

\[
C_D = \frac{S}{\frac{1}{2} \rho V^2 \text{(proj. area)}},
\]

If we use \( V_M \) for \( V \), we find that \( C_D \) increases rapidly with decreasing speed of blood flow. Thus, it is not convenient to use \( C_D \) in the preceding analyses. The term \( C_s \), which is used instead, becomes constant as the flow speed approaches zero.

In conclusion, it is possible to determine the interacting shear and normal stresses between a white blood cell and the endothelium when the white blood cell is sticking to the endothelial surface or rolling at a constant rate. This determination can be carried out by evaluating the flow field about the white blood cell by high-speed motion pictures and calculating the shear force on the basis of a dimensionless coefficient determined by a model experiment. In the venules of the rabbit mesentery and omentum, the shear force acting on a white blood cell is on the order of \( 10^{-5} \) dynes, which, divided by the contact area, results in a shear stress varying between 50 and 1060 dynes/cm² in the cases studied. The nonuniformly distributed normal stress, whose resultant moment resists the rolling moment of the shear force, is on the same order of magnitude. The shear stress varies over such a wide range because of the variable size of white blood cells and the variable concentration (hematocrit) of the blood cells. The local hematocrit in the venule varies between 0% and about 40%. The lowest shear stress is estimated on the basis of zero hematocrit, and the highest shear corresponds to the highest hematocrit.

Technically, the greatest possible error in the present approach lies in the imperfect simulation of the elasticity of the red blood cells. Our gelatin pellets are probably too rigid compared with real red blood cells.

References
Vascular endothelium-leukocyte interaction; sticking shear force in venules.
G W Schmid-Schoenbein, Y C Fung and B W Zweifach

Circ Res. 1975;36:173-184
doi: 10.1161/01.RES.36.1.173

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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