Low-Stress Hemolysis in Laminar Blood Flow: Bulk and Surface Effects in Capillaries

Human blood (blood bank, expired) sheared through stainless steel capillary tubing (508 μm ID) is analyzed for plasma hemoglobin to study the effect of shear-induced blood damage within the low-stress regime (stress $\leq$ 20 Pa). Blood damage results are described in terms of capillary length (related to blood residence time) and wall shear rate for assessing bulk and surface effects. A phenomenological model is proposed to explain these experimental results, obtained over a shear rate range to 7,000 s^{-1}.

R. L. Beissinger, Jean-Francois Laugel
Chemical Engineering Department
Illinois Institute of Technology
Chicago, IL 60616

Introduction

There is considerable research evidence (Gaarder et al., 1961; Stormorken, 1971; Myhre and Rasmussen, 1969; Myhre et al., 1970; 1971; Reimers et al., 1984) to suggest that low-stress damage of blood in contact with the synthetic surfaces of artificial organs, prosthetic devices, and extracorporeal flow systems is often associated with hemolysis via red blood cell loss of components, which include adenosine diphosphate (ADP), and thereby influences thrombosis through potentiation of platelet aggregation (Reimers et al. 1984) and adhesion (Turitto and Weiss, 1980). Therefore, a much improved knowledge of hemolysis and how it affects thrombosis is needed concerning the effects of synthetic material and mechanical trauma on various blood elements, especially red blood cells (RBC). An improved understanding of shear-induced hemolysis will add toward the overall development of biomaterials so that in clinical circumstances device materials can be better chosen and device hydrodynamics better designed to minimize hemolysis. Low-stress hemolysis (LSH)—the loss of hemoglobin (Hgb) (along with other components) from the red blood cell into the surrounding plasma—implies major cell damage, because Hgb molecules are too large to pass through the cell membrane unless the membrane is greatly deformed or ruptured; the effects are enhanced with transfused blood that has aged in blood bank storage. If severe enough, LSH induces anemia and may cause saturation of the kidney with Hgb; free Hgb concentration levels in blood plasma (greater than 160 mg/100 mL) have been noted to be toxic to the body (Blackshear, 1972).

Specifying engineering design requires an understanding of the effects of fluid shear rate and shear stress as well as device material effects on flow-induced hemolysis. However, it had never been shown until recently (Beissinger and Williams, 1984), that blood damage in one device could be predicted quantitatively from that in another. Experimental results (Shapiro and Williams, 1970) show that LSH for fixed time-shearing correlates with shear rate rather than with shear stress (Leverett et al., 1972) and has been postulated to occur at the blood-artificial surface interface (Blackshear, 1972); correlations with material chemical parameters have been found (Monroe et al., 1980; Offeman and Williams, 1979a). Also, the geometric parameters of the device used can influence hemolysis independently of wall contact and these interactions are volume- (bulk-) related. Under these conditions, correlations of flow-induced hemolysis with shear stress (Leverett et al., 1972; Sutera and Mehrjardi, 1975) and shear rate (Richardson, 1974) have also been found. Earlier findings (Offeman and Williams, 1976) have also shown that blood damage, specifically hemolysis, in low-stress shear flow depends on blood storage age. All of the above studies were comprehensively reviewed in a previous report (Beissinger and Williams, 1984). It was also found that the rheological properties of whole blood, i.e., the steady and complex viscosity, although affected by blood storage age do not correlate with hemolysis (Beissinger and Williams, 1985).

Recent results for LSH have confirmed the earlier findings as well as extended them (Beissinger and Williams, 1984). However, blood damage results for shear stress $\leq$ 13 Pa showed for blood bank expired bloods only a weak sensitivity to surface effects, with the major source of LSH occurring in the bulk fluid flow. These apparently contradictory results, as summarized in Table 1, were explained by postulating that surface and bulk damage mechanisms act in series with a relative importance that depends on blood chemistry and surface material chemistry, hydrodynamics of the flow system tested, and time of shear-

Correspondence concerning this paper should be addressed to R. L. Beissinger.

Table 1. Effect of Shearing Experience on Hemolysis

Type of Experience	Time of Shearing min	Range of Shearing Pa	Hemolysis Observed	Comments
Couette rotational flow (CRF)	2	50–400	High-stress regime, shear stress >150 Pa, nearly instantaneous rupture of RBC in bulk flow. Low-stress regime (slow leakage of Hgb from RBC) shear stress <150 Pa, is device-wall dependent and correlated with shear stress/shear rate at the wall.	Leverett et al. (1972); human blood
CRF	3	≤60	Low-stress hemolysis correlated with wall shear rate (rather than with shear stress) and time of shearing.	Shapiro & Williams (1970); human blood (stored)
CRF	5	10–450	High-stress (regime) hemolytic fragmentation of RBC in bulk occurring for shear stress >250 Pa.	Sutera & Mehrjardi (1975); human blood
Parallel disk torsional flow (PDTF)	≤80	≤45	Low-stress hemolysis correlated with chemical and physical nature of surface material (i.e., critical surface energy) and time of shearing.	Lampert & Williams (1972); human blood (stored)
PDTF	≤10	≤13	Low-stress hemolysis correlated with chemical and physical (roughness, critical surface energy) nature of surface material and time of shearing.	Offeman & Williams (1979a,b); human blood (stored)
PDTF	10	≤13	Low-stress hemolysis correlated with donor blood chemistry (including changes during storage), shear rate (to $5{,}000^{-1}$), and surface and bulk effects. However, bulk effects are most significant.	Beissinger & Williams (1984); human blood (stored)
Capillary Pouseulle flow (CPF)	<1 s	500–1,000	High-stress (regime) hemolysis correlated with capillary wall material.	Bacher & Williams (1970); bovine blood
CPF	≤130 (recirculating flow)	<2	Low-stress hemolysis correlated with surface roughness.	Wielogorski et al. (1976); bovine blood

ing. This latter mechanistic interpretation of LSH provided the theoretical basis for evaluation of the blood damage results found in this study.

This study focuses on hemolysis in smooth stainless steel (SS) capillary tubing for two expired bloods (both 25 days old), with low shear levels (1–20 Pa) occurring at the tubing walls; the effects of surface roughness on hemolysis were not evaluated in this study. These flow conditions are clinically relevant, as far as the shape of the tube (similar to the shape of blood vessels) and the magnitude of wall shear stress, shear rate and blood flow rate are concerned. The work is separated into two parts:

1. An experimental study of low-stress, shear-induced hemolysis measured in a capillary flow system.

2. A theoretical analysis of the blood damage phenomenon as a function of capillary length (this corresponds to blood residence time in the flow device), system hydrodynamics, and mass transfer.

The main objective was to evaluate the contribution of both surface and bulk effects on low-stress, shear-induced hemolysis during capillary flow under conditions of short wall contact time using the phenomenological model developed herein.

Experimental

Apparatus

A specially designed capillary viscometer used in the experiments is discussed completely in another report (Laugel, 1982). The capillary flow system was composed of a SS pressure vessel, SS capillary, and a nitrogen cylinder, Figure 1. The inlet of the capillary tubing was attached to the side of a SS vessel by a series of Swagelock connections. The exit of the capillary was connected to a Luer-Lock syringe in order to collect the blood samples immediately after their shearing experience. The top of the SS reservoir was connected to a high-pressure nitrogen cylinder that supplied the driving force for blood flow through the tubing. A pressure regulator controlled the pressure drop experienced by the blood as it flowed through the capillary tubing. Nitrogen gas was chosen in these experiments because of its

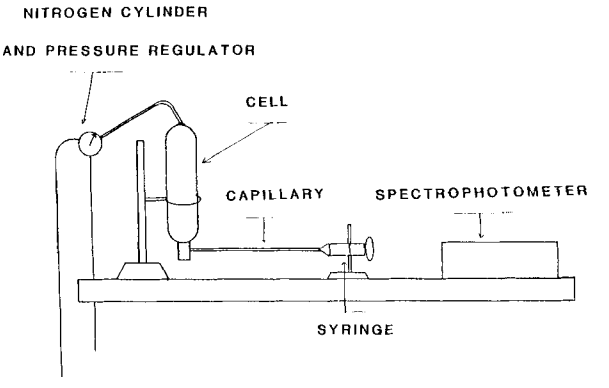

Figure 1. Capillary flow system.

negligible effect on blood damage as compared to other gases (Offeman and Williams, 1979a,b). For all pressure operating conditions used the effect of the changing hydrostatic head of blood in the blood reservoir tank negligibly affected the overall system pressure drop.

In view of a previous report (Blackshear, 1972) that temperature does not appear to be an important variable affecting blood damage, little attempt was made to keep the blood temperature constant for all experiments. However all experimental runs were made in a closed room at ambient conditions, approximately 23°C, varying by no more than ±2°C.

The capillary tubing used for the blood damage experiments was of several lengths: 1.5, 2.5, 4.0, and 10.0 m; all tubings were of the same diameter, and made of the same material, type 316 stainless steel. These tubes were high-performance liquid chromatography (HPLC) capillaries with an average radius of 254 μm and were specified by the supplier (Alltech, Waukegan, IL) as being within 10% of the nominal value.

Because of the large length-to-diameter ratios, $L/D > 6{,}000$, for even the smallest capillaries used, fully developed laminar flow of blood was assumed to occur throughout the tube flow and the imposed pressure gradients were assumed free of entrance and exit effects. This latter assumption was verified and discussed elsewhere (Laugel, 1982). The fully developed steady-flow laminar hydrodynamics are well known for non-Newtonian fluids (Bird et al., 1960, 1977; Walters, 1975). For the range of Reynolds number used, $Re < 1$, the entrance length L_e necessary for development of the centerline velocity to within 99% of its fully developed value in a circular cross section was described by (Atkinson et al., 1966):

$$\frac{L_e}{D} = 0.059 + 0.056\, Re \tag{1}$$

where D is the tube diameter. Values of $L_e/D < 0.65$ were predicted for the conditions of this experimental study, corresponding to L_e values smaller than 0.02% of the total tube length.

Materials

All blood samples tested were obtained either from the Rush Presbyterian St. Luke's Hospital Blood Bank, or from the Michael Reese Hospital Blood Bank, both located in Chicago. The samples were collected from healthy donors and had normal hematocrit, ranging from 35 to 45%. The blood samples used ranged in storage age, but were never less than 10 days nor more than 25 days old. They had been preserved in sterile bags using citrate phosphate dextrose adenine (CPDA). Until their use, the blood bags were stored at 4°C in a laboratory refrigerator, but just prior to an experiment the blood samples were equilibrated to room temperature. This equilibration usually took approximately 40 min.

Methods

Before each experiment all surfaces of the capillary flow system coming in contact with blood during the experiments were cleaned in phosphate detergent (Sparkleen, Fisher Scientific), then rinsed initially with distilled water and finally with an isotonic phosphate buffered saline solution (pH 7.4). About 400 mL of blood were then carefully transferred into the clean test vessel by slow gravity flow through the blood bag tubing. During the filling operation about one-third of the blood sample was continuously withdrawn from the test vessel and discarded. After the bottom third of the blood sample had been discarded, two reference samples were withdrawn. The gas cylinder was then connected to the test vessel and the system was pressurized to the prescribed level. The appropriate capillary tubing to be used for testing was connected, then a flow valve was opened and blood instantly began to flow through the capillary. To ensure that the test samples to be collected were sheared at the prescribed shear stress level in the flow system, a small portion of blood was first sheared at the appropriate test conditions for a period of three residence times (average time spent by the blood in the capillary volume) and discarded. Then, immediately, a syringe was connected to the flow line and three 4 mL samples of sheared blood were collected. All three samples nearly always gave about the same blood damage results, ensuring that steady state conditions were being measured. Both the time elapsed during which blood was collected and the blood volume collected in the syringe were recorded. Part of the blood sample was used to determine the hematocrit. The remaining portion was centrifuged for 10 min in a standard clinical centrifuge at 1,200 g acceleration. The plasma supernatant was then withdrawn and analyzed for Hgb. The approach followed is described elsewhere (Laugel, 1982) and is based on a tetramethylbenzidine reaction following methods developed by Standefer and Vanderjagt (1977).

Determination of Rheological Properties. The capillary flow system was used to determine the apparent viscosity η of the blood samples as a function of steady shear rate $\dot{\gamma}$ and shear stress τ (Bird et al., 1977). Wall shear stress values τ_R were determined from the steady shear flow pressure gradient.

$$\tau_R = \frac{\Delta P\, R}{2L} \tag{2}$$

Wall shear rate values were obtained by determining the slope from plots of $Q\tau_R^3/\pi R^3$ vs. τ_R and then evaluating the expression

$$\dot{\gamma}_R = \left[\frac{1}{\tau_R^2}\frac{d(Q\tau_R^3/\pi R^3)}{d(\tau_R)}\right] \tag{3}$$

Blood viscosity values were determined by evaluating the ratio of wall shear stress to the wall shear rate

$$\eta = \frac{\tau_R}{\dot{\gamma}_R} \tag{4}$$

These expressions were used with the measured experimental data of the form $Q(\Delta P)$. For a given experimental run using the syringe collection device connected to the end of the capillary, the volumetric flow rate was easily determined.

Measurement of LSH. All hemolysis experiments were performed with several different samples of normal whole blood in which the hematocrit did not vary appreciably (35 to 45%). Therefore, a convenient quantity was defined for comparison of blood damage of all the bloods tested. Hemolysis, H, is defined

as

$$H = \frac{\bar{C}_H^* - \bar{C}_{HO}^*}{\bar{C}_{HO}^*} = \frac{\bar{C}_H^*}{\bar{C}_{HO}^*} - 1 \qquad (5)$$

where $\bar{C}_H^*$ is the blood plasma Hgb concentration of the sheared sample and $\bar{C}_{HO}^*$ is the blood plasma Hgb concentration of the unsheared reference sample. By definition $H = 0$ when no hemolysis has occurred during flow.

Although most previous studies have postulated that blood damage in the low-stress regime occurs at or near the solid artificial surface and is most appropriately correlated with either $\dot{\gamma}_R$ or τ_R, results from a recent study (Beissinger and Williams, 1984) indicate a contribution of both surface and bulk effects on hemolysis. Therefore, it has been attempted to distinguish experimentally between these two effects in capillary flow by describing blood damage as a function of:

1. Parameters associated with bulk effects, i.e., tube residence time t_r or capillary flow length L, and
2. Parameters associated with surface effects, i.e., $\dot{\gamma}_R$ or τ_R, both of constant value along the capillary tube wall.

Results

Viscosity

Viscosity measurements were made for all the blood samples tested. Typical results for blood-bank stored bloods are shown in Figure 2, where viscosity is plotted as a function of shear rate. For $\dot{\gamma} > 2{,}500\ s^{-1}$ blood viscosity η appears to be approximately constant with a value of about 2.7 mPa.s at 23°C.

Blood damage

The first part of the blood damage investigation deals with the effect of residence time on LSH for several values of wall shear stress, Figure 3. Note that L changes as t_r is varied because these parameters are related ($t_r = \pi R^2 L/Q$). As expected, hemolysis (the dimensionless plasma hemoglobin concentration) H increases as t_r increases, for a given τ_R. Also, damage increases for a given residence time as τ_R increases. Hemolysis is seen to increase with t_r most rapidly initially, and then to increase more slowly thereafter.

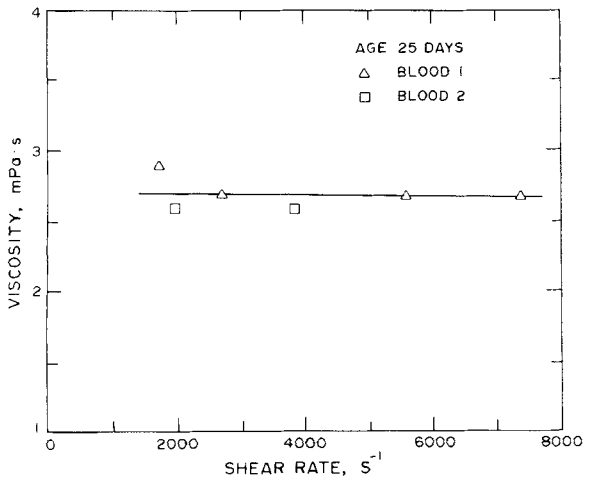

Figure 2. Non-Newtonian viscosity $\eta(\dot{\gamma})$ obtained in capillary flow system, capillary radius 250 μm.
Shear rate range was sufficiently high with blood showing nearly Newtonian behavior.

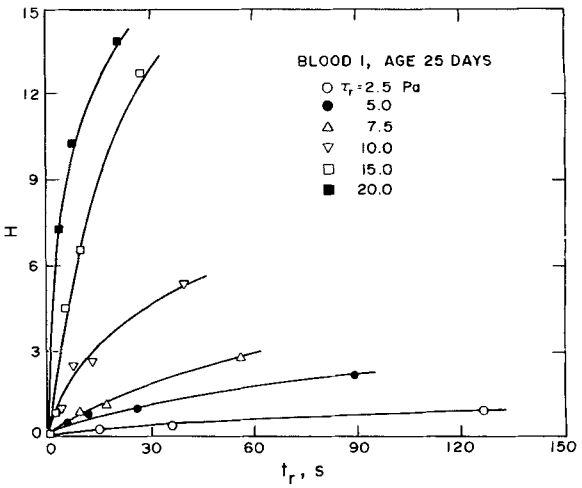

Figure 3. Change of dimensionless plasma hemoglobin concentration H with capillary residence time for several values of wall shear stress.

Experimental results on the effect of capillary length L on low-stress hemolysis for a given $\dot{\gamma}_R$ are shown in Figures 4 and 5 for bloods 1 and 2. Because t_r and L are related, it is expected that Figures 3, 4, and 5 should be similar. As Figures 4 and 5 show, this turns out to be the case, with the data exhibiting a rapid increase in H for the smaller lengths, followed by reduction in curvature for the larger ones. This effect was more pronounced for the higher $\dot{\gamma}_R$ runs. Although $\dot{\gamma}_R$ and τ_R are rheologically related, experimental results obtained in another study (Shapiro and Williams, 1970), show that LSH correlates with $\dot{\gamma}_R$ rather than with τ_R. Therefore $\dot{\gamma}_R$ was chosen as a more appropriate surface parameter for blood damage correlation.

Although one diameter of capillary tubing was used in this study (504 μm), capillary tubing length varied from 1.5 to 10.0 m. An important result shown in Figure 4 is that the blood damage occurring within the first 1.5 m of capillary is surprisingly low (based on extrapolations of the smooth curves to the abscissa) and a threshold (delay) length L_o can be defined below which

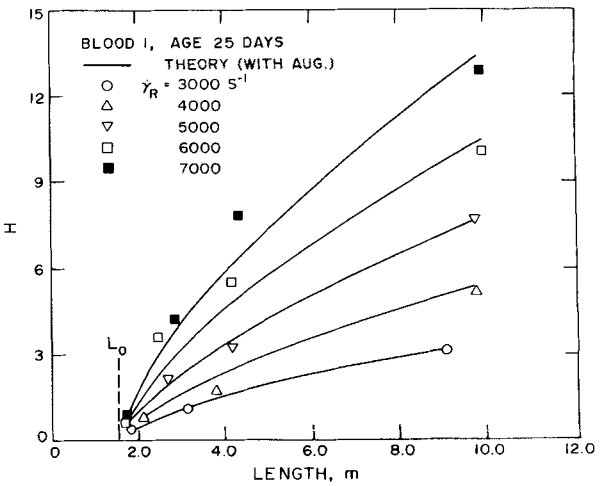

Figure 4. Measured change of dimensionless plasma hemoglobin concentration H with increasing capillary length for Blood 1.

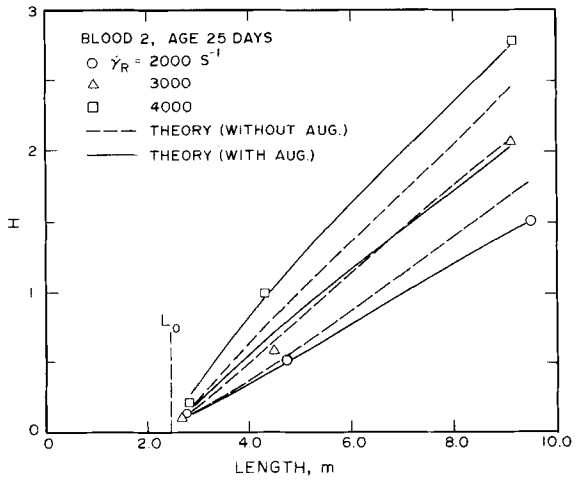

Figure 5. Measured change of dimensionless plasma hemoglobin concentration *H* with increasing capillary length for Blood 2.

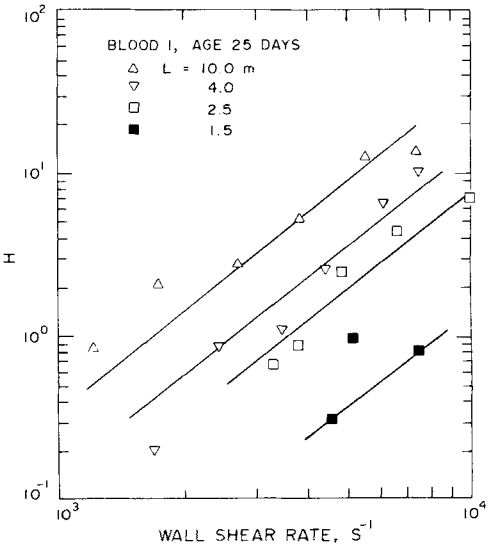

Figure 6. Change of dimensionless plasma hemoglobin concentration *H* with increasing wall shear rate for four different capillary tubing lengths.
Lines are not a least-squares fit, but are drawn by eye with slopes of 2.

hemolysis is negligibly small. However, it appears that L_o may depend slightly on $\dot{\gamma}_R$. As noted previously, bulk-flow hydrodynamic entrance length effects are negligible ($L_e \leq 0.65D = 0.3$ mm) and therefore probably not responsible for L_o, since the velocity profile is fully developed throughout even the smallest capillary length used in these experiments.

The delay length can also be interpreted as a corresponding delay time, t_o, i.e., the time in which it takes blood very near the walls of the tube to flow from the entrance of the capillary a length equivalent to L_o. That $H(L)$ shows a delay length L_o in Figure 4 (blood 1), but $H(t_r)$ shows no delay residence time t_{ro} in Figure 3, is at first glance disconcerting, since $t_r \propto L$ ($t_r = \pi R^2 L/Q$). A likely explanation why a delay residence time is not observable in Figure 3 is probably that t_r represents the average time spent to travel a capillary of length L for all red blood cells at all cross-sectional locations, t_{ro} should be considerably smaller than t_o. Therefore plots like that of Figure 3 are probably not as sensitive as those of Figure 4 to show blood damage delay effects in capillary flow.

The behavior of $H(\dot{\gamma}_R)$ for given lengths of capillary tubing is shown for two different bloods in Figures 6 and 7. Both bloods had been obtained from healthy donors and stored 25 days prior to testing. These log-log plots suggest that $H(\dot{\gamma}_R)$ has a power law dependence on $\dot{\gamma}_R$,

$$H \propto \dot{\gamma}_R^n \tag{6}$$

with slope n approximately equal to 2 in Figure 6 and equal to 1 in Figure 7. The power law functional form used in the correlation is consistent with the form used in a previous report (Beissinger and Williams, 1984) where the exponent was found to have a value of 1.22 for the blood tested. The difference in n values between the two bloods indicates a difference in their hemolytic susceptibility to shear. However no firm conclusions should be drawn from these comparative figures, since the shear rate range over which blood 2 was tested, 2,000–4,000 s^{-1}, is too small and contains too few data points per curve to establish the exponent of the power law function reliably. Actually, the experimental blood damage data plotted in Figures 4 and 5 show that hemolysis values H for blood 1 are about 50% greater than for blood 2 for wall shear rates of 3,000 and 4,000 s^{-1}. Also as noted above, the extrapolated L_o is larger for blood 2 (2.5 m) as compared to blood 1 (1.5 m). If L_o can be used as an experimental parameter to assess the susceptibility of a blood to be damaged by low-stress shearing, then the extrapolated values indicate blood 2 is less hemolytically fragile than blood 1.

The experimental results reported in this section provided the basis for the development of a phenomenological model to explain low-stress, shear-induced hemolysis in capillary flow. According to the work of others (O'Rear et al., 1972; Shapiro and Williams, 1970) and the experimental results discussed

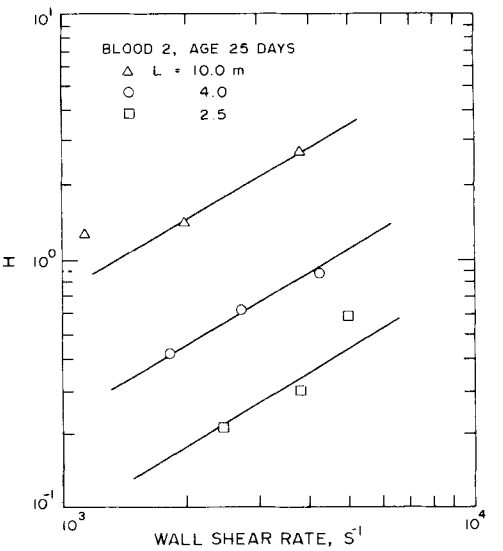

Figure 7. Change of dimensionless plasma hemoglobin concentration *H* with increasing wall shear rate for three different capillary lengths.
Lines are not a least-squares fit, but are drawn by eye with slopes of 1.

herein, Figures 6 and 7, shear rate seems to be a relevant parameter for correlating hemolysis. Furthermore, it appears that the length of capillary tubing, which represents the distance the red blood cells travel, is also an appropriate parameter influencing steady state blood damage phenomena. Therefore, hemolysis was modeled primarily as a function of these two variables, $\dot{\gamma}_R$ and L, and L_o was incorporated empirically into the theoretical model.

Theoretical Model

Development

The development of the model will only be highlighted here. A detailed description of the model is reported by Laugel (1982) as well as in the Supplementary Material. (See the footnote at the end of the text.) To describe the generation of free hemoglobin (Hgb) from damaged red blood cells (RBC), the convective diffusion equation (Bird et al., 1960), with appropriate boundary conditions, was used to model the behavior of three blood elements flowing in the capillary tubing system: undamaged red blood cells (URBC), damaged red blood cells (DRBC), and plasma hemoglobin molecules released from the DRBC into the plasma. For this idealization it was assumed that only these species were relevant in the hemolytic process. The flow behavior of blood was considered to be Newtonian, to a first approximation, consistent with the viscosity-shear rate behavior presented in Figure 2.

The assumption of short wall contact time (Bird et al., 1960; Leveque, 1928) was made for the flow of URBC and DRBC described by large Peclet number (*Pe*) and small red diffusivity. The value of the cell effective diffusivity at normal hematocrit (40%) in laminar shear flow was extrapolated to be on the order of 10^{-7} cm^2/s from other studies involving considerably smaller shear rates (Goldsmith and Marlow, 1979; Eckstein et al., 1977) than used in this study and for the Eckstein study, particle sizes two orders of magnitude larger than for RBC. A large RBC diffusivity value is expected even near the capillary wall for normal hematocrit since the inward migration of RBC would be hindered and the peripheral cell-reduced layer would be too small to prevent wall encounters of RBC (Goldsmith and Marlow, 1979). Using diffusivity values of 10^{-7} cm^2/s for both URBC and DRBC satisfies the assumption of short wall contact time as demonstrated elsewhere (Laugel, 1982; Supplementary Material). Thus, significant changes in the DRBC and URBC concentration profiles are limited to a thin boundary layer, $\delta(z)$, adjacent to the wall. Therefore, the capillary flow of both URBC and DRBC was approximated by flow over a flat plate under steady state conditions using the Leveque approximation; the well-known dimensionless equations governing the mass transfer behavior are shown elsewhere (Laugel, 1982; Bird et al., 1960; Supplementary Material).

The solution of these equations is obtained analytically, as shown elsewhere (Bird et al., 1960). In particular, the solution is used to give the flux of URBC to the capillary wall

$$-D_u \left.\frac{\partial C_u}{\partial y}\right|_{y=0} \cdot C^*_{uo} = \frac{D_u C^*_{uo}}{\Gamma(4/3)} \cdot \left(\frac{|\dot{\gamma}_R|}{9D_u z}\right)^{1/3} \tag{7}$$

where y is the actual distance from the wall, D_u the URBC diffusivity, $\dot{\gamma}_R$ the wall shear rate, z the modified axial distance down the tube, C_u the dimensionless URBC concentration, C^*_u the local URBC concentration and C^*_{uo} the initial URBC concentration at the entrance to the capillary, and $\Gamma(4/3)$ is the gamma function. Verification to show that the flat-plate approximation is satisfactory for the various L and $\dot{\gamma}_R$ experimental conditions used in this study is given elsewhere (Laugel, 1982; Supplementary Material).

The dimensionless DRBC concentration C_d is obtained by using a conservation of (all) cells principle,

$$C_u + C_d = C_T \tag{8}$$

where C_T represents the total concentration of all cells.

The modeling of the convection, diffusion, and generation of plasma hemoglobin, resulting from damage of the red blood cells as described above, is considerably more involved. The Leveque model (transport much slower than "reaction" at the wall) is no longer valid for hemoglobin molecules, as the effective diffusion coefficient D'_H can be considerably larger than that used for red blood cells, about 10^{-6} cm^2/s (Tanford, 1961), and also a generation term appears in the bulk. Therefore the steady state convection diffusion equation to account for plasma Hgb transport across the entire capillary cross section is

$$v_z(r)\frac{\partial C^*_H}{\partial z} = D'_H\left[\frac{1}{r}\frac{\partial}{\partial r}\left(r\frac{\partial C^*_H}{\partial r}\right)\right] + K_1 C^*_d|\dot{\gamma}_r|^G \tag{9}$$

with boundary conditions

$$\text{at} \quad z = 0, \quad C^*_H - C^*_{Ho} = 0 \tag{9a}$$

$$\text{at} \quad r = 0, \quad \frac{\partial C^*_H}{\partial r} = 0 \tag{9b}$$

$$\text{at} \quad r = R, \quad D'_H\frac{\partial C^*_H}{\partial r} = -D_u\frac{\partial C^*_u}{\partial r}K_2|\dot{\gamma}_R|^G \tag{9c}$$

where r is the radial distance, R is the capillary radius, C^*_H is the plasma hemoglobin concentration, D'_H is the effective diffusivity of hemoglobin, $v_z(r)$ is the axial velocity of the fully developed flow, and $\dot{\gamma}_r$ is the shear rate at a specified radial location.

In Eq. 9 generation of hemoglobin in the bulk fluid was related to the concentration of all DRBC, newly damaged or not, and was represented by a production term assumed to be proportional to the concentration of damaged cells C_d that continue to leak at a steady rate. Furthermore, the hemoglobin generation in the fluid bulk and at the wall were assumed to have a functional dependence (similar to that determined experimentally in this study, see Figures 6 and 7) on shear rate at the fluid streamline considered raised to some appropriate power represented by G in Eq. 9 and 9c. Notice that Eq. 9c represents the generation of hemoglobin at the capillary wall, where the flux of plasma hemoglobin away from the wall is assumed to be proportional to the magnitude of the wall shear rate raised to the G power, $(|\dot{\gamma}_R|)^G$, as well as to the net flux of URBC to the tubing wall to be damaged. Two additional phenomenological parameters have also been introduced in the equations describing plasma hemoglobin transport: K_1 and K_2 are rate constants for bulk and surface-related blood damage, respectively.

The effective diffusion coefficient of hemoglobin D'_H was not taken to be constant in this capillary flow system, but to depend on shear rate ($\dot{\gamma}_r$). Several previous workers (Turitto et al., 1972; Collingham, 1968; Grabowski et al., 1972; Antonini et al., 1978) have found the diffusion coefficient of small particles and

macromolecules in flowing suspensions to increase as the level of shearing increases. The work of Wang and Keller (1985) on augmented transport in sheared suspensions was useful in the development of Hgb transport modeled here. Their correlation-based approach for finding diffusivity augmentation with shear rate (up to three orders of magnitude in this study) was followed in determining the appropriate D'_H value to be used in Eq. 9. The details of the method used are reported elsewhere (Laugel, 1982; Supplementary Material).

For a given set of phenomenological parameters, K_1, K_2, and G describing the experimental blood damage behavior, Eq. 9 was solved numerically by finite-difference (Von Rosenberg, 1977) using an implicit Crank-Nicholson procedure. The complete description of the numerical method used is discussed in an earlier report (Laugel, 1982).

To compare the predicted theoretical results of blood damage with the experimental data, the experimentally measured bulk cup-mixing concentration of plasma hemoglobin $|C_H|$ (Bird et al., 1960), which is represented nondimensionally, was computed:

$$\langle C_H(L) \rangle = 4 \int_0^1 C_H(1 - \phi^2)\phi d\phi \qquad (10)$$

where $\phi = r/R$. Then $\langle C_H \rangle$ was determined for various capillary lengths by numerical integration of Eq. 10 using Simpson's rule.

Values of the phenomenological parameters K_1, K_2, and G used in the nonlinear blood damage model to describe the red blood cell hemolytic behavior are reported in Table 2 and were estimated from the experimental data by least-squares, using a pseudo-Gauss-Newton algorithm (BMDP, 1982).

Fits of the blood damage model to the $H(L)$ data, using the parameter values listed in Table 2, are shown in Figure 4 for blood 1 and in Figure 5 for blood 2. When the model for blood 2 was simplified by setting the bulk damage parameter K_1 equal to zero, resulting in a surface-damage model only, the standard deviation of the residuals was more than five times greater than that for the three-parameter bulk-surface model, i.e., 0.144 vs. 0.025. Also shown in Figure 5 for blood 2 by dashed lines are model predictions without augmentation of hemoglobin diffusivity with shear rate. For this case the standard deviation of the residuals for the three-parameter model without augmentation is about one order of magnitude larger than the three-parameter model that includes hemoglobin diffusivity augmentation. In Figure 8 at the $H(\sigma)$ data are fitted as a function of axial position [where $\sigma = (z' - L_o)/(L' - L_o)$] via the model through Eq. 10.

When comparing different bloods for the character of their hemolytic sensitivity a relative damage number parameter, K, was defined

$$K = K_1/K_2 \qquad (11)$$

Table 2. Best Set of Parameter Values for Bloods 1 and 2

Parameter	Blood 1	Blood 2
$\dot{\gamma}_R$ Range, s^{-1}	3,000–7,000	2,000–4,000
K_1, $(mg/cell)s^{G-1}$	4.20×10^{-8}	3.76×10^{-6}
K_2, $(mg/cell)s^{G}$	10.63×10^{-6}	6.18×10^{-6}
G	1.35	1.31
K, s^{-1}	0.004	0.61

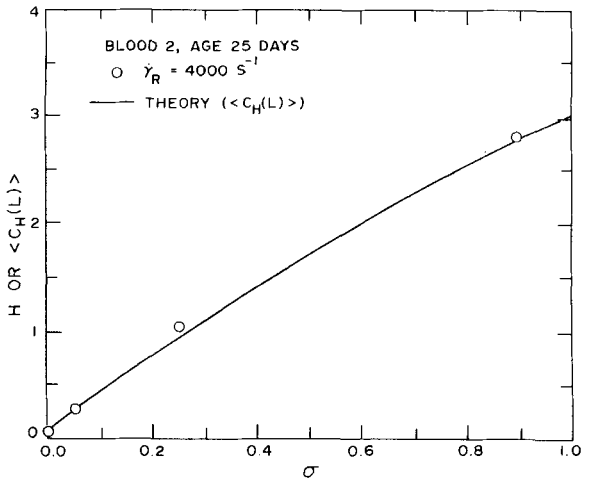

Figure 8. Comparison of dimensionless plasma hemoglobin concentration H with prediction of the dimensionless cup-mixing hemoglobin concentration.

Both concentrations are represented in terms of dimensionless axial position.

where K has units of reciprocal seconds. K represents the ratio of a blood's bulk-damage rate constant to surface-damage rate constant and is a function of blood chemistry and surface material properties, which include physical and chemical factors. Comparing the relative damage number of blood 1, $K = 0.004$ s^{-1}, to that of blood 2, $K = 0.61$ s^{-1}, indicates that blood damage in the bulk occurs to a substantially greater extent for blood 2. Surface damage rates for the two bloods are comparable; the distinctions appear with bulk damage rates.

Discussion of Model and Results

The blood damage model proposed in this study bears a generic relationship to a model proposed by Keller and Lauffenburger (1978), i.e., both include the effects of surface on blood damage. However, their model involves cell transport from a core region across a film (not a boundary layer as considered herein), defined as the "skimming layer" of reduced cell density near the wall. Although their model was more general and could be applied to different types of blood damage phenomena as compared to the present study, it was:

1. Not as helpful for dealing with the combination of surface and bulk effects on blood damage, and
2. Not based on experimental blood damage data.

At hematocrit levels above 35% it seems appropriate to treat the capillary blood flow in this study as macroscopically homogeneous, i.e., with no RBC-depleted layer adjacent to the wall, and to use the simplified model described herein for predicting RBC transport to and from the capillary wall. As discussed in the Supplementary Material, several studies support this interpretation (Goldsmith, 1972; Cokelet, 1972; Diller et al., 1980; Bloch, 1962; Bugliarello and Hayden, 1963; Phibbs, 1968).

The overall fit of the model to the experimental measurements H and the dimensionless hemoglobin cup-mixing concentration $\langle C_H \rangle$ appears satisfactory for both bloods. However, blood 2 appears visually to be better fitted by the model than blood 1, Figure 4, 5, 8.

Two assumptions used in the development of the model equations were examined as sources of problems and shown to be satisfied as reported elsewhere (Supplementary Material):

1. Isothermal flow in the capillary, i.e., no viscous dissipation
2. Short wall contact time for the red blood cells, i.e., the steady state Leveque model (Bird et al., 1960; Leveque, 1928).

For the conditions of short wall contact time controlling, as in this study, the resulting hemoglobin concentration being produced in the flowing blood is dependent on capillary wall effects. This is expected since surface-induced trauma occurs as long as undamaged red blood cells exist in the flowing blood. However, in the situation where shear rate is high and wall contact time is long, virtually all the red blood cells will have been surface-traumatized, resulting in hemolysis controlled by bulk flow conditions only. In clinically relevant flows, such as those occurring in artificial organs and extracorporeal systems, the entire range of short to long wall contact time flow episodes is possible.

In a recent benchmark study (Beissinger and Williams, 1984), low-stress hemolysis results, using a torsional viscometer with a surface to volume (S/V) range of 2 to 8 mm^{-1}, showed a weak sensitivity to solid surface effects, with the major source of blood damage being in the bulk fluid. In that study the degree to which both surface and bulk effects contribute was assessed through correlation only. However in this study the phenomenological model developed implied that the hemolytic damage for bloods 1 and 2 for an S/V value of 8 mm^{-1} was much more sensitive to surface effects than bulk effects. Model parameters found for both bloods, given in Table 2, show that surface effects contributed over 99% of the blood damage for blood 1 at $\dot{\gamma}_R$ = 4,000 s^{-1} and L = 10.0 m, whereas surface effects were only responsible for 74% of the total damage for blood 2 at $\dot{\gamma}_R$ = 4,000 s^{-1} and L = 10.0 m. However, blood residence times in the torsional viscometer of the benchmark study, 10 min, were about 5 to 100 times greater, as shown in Figure 3, than those occurring in this study's capillary flow system. Therefore in accordance with the series model, blood damage in the bulk fluid would have greater importance for longer blood residence times, as is the case for the earlier study, compared to the present study. Also, as was noted in the other work, the leakage of Hgb would be dependent on S/V as long as surface-induced cell traumas were a continuing process while the flow was sustained. However for long residence times and high shear rates (as first argued by Blackshear, 1972) the S/V dependence would be lost after all the URBC had made contact with the solid surface, resulting in their damage. Then low-stress hemolysis would continue in the bulk, but only as a function of bulk flow conditions.

As Table 2 above indicated, blood 1 and blood 2 showed substantially different blood damage behavior, although their rheological properties were similar, Figure 2. This result is consistent with that found in another study on the effects of blood storage on rheology and damage in low-stress shear flows (Beissinger and Williams, 1985).

Possible mechanisms of surface-induced shearing damage to blood include the detrimental chemical effects of the artificial surface contacting the red blood cell membrane. Also as mentioned in the previous study, sliding friction along a solid wall would create high membrane stress and deformation, allowing even large molecules to escape through locally enlarged pores. In addition, membrane contact with the walls, even momentarily, could result in tether formation, a region of great membrane stretch and potential porosity (Beissinger and Williams, 1984), followed by rupture with leakage of cellular components. Following these surface-induced blood damage effects, bulk effects occur. One possible mechanism suggests that the leakage of hemoglobin from the damaged cells as they diffuse into the bulk is due to the development of membrane pores. The pores arise from membrane deformation as a result of bulk shearing.

The concept of a blood damage "delay length" can be interpreted as the minimum length that red blood cells must travel in a capillary before any significant leakage of hemoglobin can be detected. Cells sheared near the entrance of the capillary and along the tubing wall do not appear to release hemoglobin at that tubing location. Apparently the cells near the wall must undergo a series of collisions with the wall, starting from the entrance of the capillary and continuing a distance L_o, before the damage experienced by the cells is sufficient to cause leakage of hemoglobin through the red blood cell membrane. The delay length concept can also be viewed as the necessary preconditioning length along the capillary wall for an adsorbed layer of plasma proteins, which become denatured due to surface interactions, to promote shear-induced leakage of hemoglobin from the red blood cells. For either mechanism the release of hemoglobin is probably not instantaneous and the distance traveled before it can be experimentally detected should depend on the chemistry of the blood and the shearing conditions. In Figure 4 ($3{,}000 < \dot{\gamma}_R < 7{,}000\ s^{-1}$) L_o was about 1.5 m, while in Figure 5 ($2{,}000 < \dot{\gamma}_R < 4{,}000\ s^{-1}$) L_o was about 2.5 m.

The whole theoretical treatment of blood damage in this study has been hydrodynamic, however inlet blood trauma effects, for example extensional elements in the converging flow field, may either cause damage to the RBC or predispose them to subsequent damage. Therefore, without further investigation it is not clear whether all hemolysis is caused by steady state flow along the wall and is entirely independent of inlet conditions. Possibly the inlet blood trauma effects may be linked to delay length, L_o.

For all the mechanisms suggested, it must also be considered that it may not be the entire population of red blood cells that participate in the blood damage process. As Offeman and Williams (1979a, b) suggest, the blood damage observed may be a manifestation of the distribution of red blood cell fragilities within the sample tested.

The phenomenological model developed in this study demonstrated an understanding of blood damage in capillary flow. Further studies are planned to test the capability of such phenomenological models, based on correlation of blood damage data in one device to predict data in another. Also, to further improve the understanding of shear-induced hemolysis, flow models need to include an assessment of surface material effects. This involves the decoupling of the effects of surface chemistry from those of surface roughness.

Summary and Conclusions

Release of hemoglobin from red blood cells under laminar flow conditions could be correlated in terms of capillary flow length (related to blood residence time in the shearing device), wall shear stress, and shear rate. The erythrocytes traveled a minimum capillary distance, a delay length, before any signifi-

cant loss of hemoglobin was detected. The delay length result could not be explained in terms of a cell-free layer near the capillary wall because at the physiological hematocrit levels used in this study, a red blood cell-free layer does not exist near the capillary wall and there is no radial migration of RBC toward the center of the capillary.

The phenomenological model, developed herein and based on the assumptions that contact (or residence) time of blood in the capillary flow system is short and surface and bulk damage mechanisms for blood damage act in series, provided an explanation of the hemolytic results that implied blood damage was more sensitive to surface effects than bulk effects. However, in the situation where wall contact (or residence) time is long, as in the benchmark study (Beissinger and Williams, 1984), nearly all erythrocytes in the flow device will have been surface-traumatized, resulting in further blood damage occurring mainly in the bulk fluid.

Acknowledgment

We are grateful to M.C. Williams of the University of California at Berkeley, for several helpful suggestions and to T. Alkhamis (Illinois Inst. of Technology) for his assistance in preparing the figures in the manuscript.

Notation

$\bar{C}_H^*$ = measured plasma hemoglobin concentration of sheared blood sample, mg/100 mL
$\bar{C}_{Ho}^*$ = measured plasma hemoglobin concentration of unsheared blood sample, mg/100 mL
C^* = concentration in plasma at a specific radial and axial position in the capillary, mg/100 mL for hemoglobin, cells/100 mL for undamaged and damaged red blood cells.
C = dimensionless concentration, as in $C_H = (C_H^* - C_{HO}^*)C_{HO}^*$ for hemoglobin, $C_u = C_u^*/C_{uo}^*$ and $C_d = C_d^*/C_{do}^*$ for undamaged and damaged red blood cells, respectively
$\langle C_H \rangle$ = predicted dimensionless bulk cup-mixing concentration of plasma hemoglobin
D = diffusivity, cm^2/s for hemoglobin, undamaged, and damaged blood red cells
D' = effective diffusivity as it relates to hemoglobin, cm^2/s
G = exponential blood damage parameter
H = hemolytic blood damage parameter = $\bar{C}_H^*/\bar{C}_{Ho}^* - 1$
H_{CR} = hematocrit, volume fraction of red blood cells in blood sample
K = relative damage number = K_1/K_2, s^{-1}
K_1 = bulk blood damage parameter, $(mg/cell)\ s^{G-1}$
K_2 = surface blood damage parameters, $(mg/cell)\ s^G$
L = total length of capillary, m
L_e = capillary entrance length, m
L_o = capillary delay length, m
Q = flow rate, cm^3/s
R = radius of capillary, cm
t_r = tube residence time = V/Q, s
t_{ro} = tube residence time corresponding to delay length, s
t_o = delay time corresponding to time it takes blood near the capillary walls to travel the delay length L_o, s
V = tube volume, cm^3
$v_z(r)$ = axial velocity of fully developed capillary flow, cm/s
$y = R - r$ = distance from capillary wall, cm
$Y = y/R$ = dimensionless distance from capillary wall
$z = z' - L_o$ = axial distance parameter measured from delay length, cm
z' = axial distance parameter measured from tube inlet, cm

Greek letters

$\dot{\gamma}$ = shear rate in capillary flow, s^{-1}
τ = shear stress in capillary flow, Pa
η = apparent viscosity in steady shear, mPa · s or cp
Δ = difference, as in ΔP
π = 3.14159 . . .
λ = dimensionless distance from wall = $y/\delta(z)$
ζ = dimensionless axial distance = x/L
ϕ = dimensionless radial distance = r/R
σ = normalized dimensionless axial distance = $(z' - L_o)/(L - L_o)$
$\delta(z)$ = thickness of diffusion boundary layer
$\Gamma(4/3)$ = gamma function = 0.89297 . . .

Subscripts

d = damaged red blood cells
H = blood plasma hemoglobin
o = inlet value or baseline value
R = value at the capillary wall
r = value at certain radial position in capillary
T = total of all cells, i.e., undamaged red blood cells plus damaged red blood cells
u = undamaged red blood cells
z = value at certain axial position in capillary

Superscript

* = quantity with units

Literature Cited

Antonini, G., G. Guiffort, D. Quemada, and A. M. Dosne, "Estimation of Platelet Diffusivity in Flowing Blood," *Biorheology*, **15**, 111 (1978).

Atkinson, B., M. P. Brocklebank, C. C. H. Card, and J. M. Smith, "Low Reynolds Number Developing Flows," *AIChE J.*, **15**, 548 (1969).

Bacher, R. P., and M. C. Williams, "Hemolysis in Capillary Flow," *J. Lab. Clin. Med.*, **76**, 485 (1970).

Beissinger, R. L., and M. C. Williams, "A Dual Mechanism for Low-Stress Hemolysis in Laminar Blood Flow, *AIChE J.*, **30**, 569 (1984).

———, "Effects of Blood Storage on Rheology and Damage in Low-Stress Flow," *Biorheology*, **22**, 477 (1985).

Bird, R. B., R. C. Armstrong, and O. Hassager, *Dynamics of Polymeric Liquids*, **1**, *Fluid Mechanics*, Wiley, New York (1977).

Bird, R. B., W. E. Steward, and E. N. Lightfoot, *Transport Phenomena*, Wiley, New York (1960).

Blackshear, P. L., "Mechanical Hemolysis in Flowing Blood," *Biomechanics: Its Foundations and Objectives*, Y. C. Fung, N. Perrone, and M. Anliker, eds., Prentice-Hall, Englewood Cliffs, NJ (1972).

Bloch, E. H., "A Quantitative Study of the Hemodynamics in the Living Microvascular System," *Am. J. Anatomy*, **110**, 125 (1962).

BMDP Statistical Software, Inc., Los Angeles, CA (1982).

Bugliarello, G., and J. W. Hayden, "Detailed Characteristics of the Flow of Blood *in vitro*," *Trans. Soc. Rheol.*, **7**, 209 (1963).

Cokelet, G. R., "The Rheology of Human Blood," *Biomechanics: Its Foundation and Objectives*, Y. C. Fung, N. Perrone, and M. Anliker, eds., Prentice-Hall, Englewood Cliffs, NJ (1972).

Collingham, R. E., "Mass Transfer in Flowing Suspensions," *Ph.D. Thesis*, Univ. Minn. (1968).

Diller, T. E., B. B. Mikic, and P. A. Drinker, "Shear-Induced Augmentation of Oxygen Transfer in Blood," *J. Biomech. Eng.*, **102**, 67 (1980).

Eckstein, E. C., D. G. Bailay, and A. H. Shapiro, "Self-Diffusion of Particles in Shear Flow of a Suspension," *J. Fluid Mech.*, **79**, 191 (1977).

Gaarder, A., J. Jonsen, S. Laland, A. Hellem, and P. A. Owren, "Adenosine Diphosphate in Red Cells as a Factor in the Adhesiveness of Human Blood," *Nature*, **192**, 531 (1961).

Goldsmith, H. L., "The Flow of Model Particles and Blood Cells and Its Relation to Thrombogenesis, *Progress in Hemostasis and Thrombosis*, T. H. Spaet, ed., Grune and Stratton, New York (1972).

Goldsmith, H. L., and J. C. Marlow, "Flow Behavior of Erythrocytes. II: Particle Motions in Concentrated Suspensions of Ghost Cells," *J. Colloid Interface Sci.*, **71**, 383 (1979).

Grabowski, E. F., L. I. Friedman, and E. F. Leonard, "Effects of Shear Rate on Diffusion and Adhesion of Blood Platelets to a Foreign Surface," *Ind. Eng. Chem. Fundam.*, **11**, 225 (1972).

Keller, K. H., and D. Lauffenburger, "The Effect of Flow Channel Thickness on Surface-Induced Blood Damage," *J. Bioeng.*, **3,** 205 (1978).
Lampert, R. H., and M. C. Williams, "Effect of Surface Materials on Shear-Induced Hemolysis," *J. Biomed. Mater. Res.*, **6,** 499 (1972).
Laugel, J.-F., "Low-Stress, Shear-Induced Hemolysis in Capillary Flow," *MS Thesis,* Ill. Inst. Tech. (1982).
Leveque, M. A., "Laws of Heat Transmission by Convection," *Ann. Mines,* **13,** 201 (1928).
Leverett, L. B., J. D. Hellums, C. P. Alfrey, and B. C. Lynch, "Red Blood Cell Damage by Shear Stress," *Biophys. J.*, **12,** 257 (1972).
Monroe, J. M., R. C. Lijana, and M. C. Williams, "Hemolytic Properties of Special Materials Exposed to a Shear Flow, and Plasma Changes with Shear," *Biomat., Med. Dev., Art. Organs,* **8,** 103 (1980).
Myhre, E., and K. Rasmussen, "Mechanical Hemolysis in Aortic Valvular Diseases and Aortic Ball Valve Prosthesis," *Acta Med. Scand.*, **186,** 543 (1969).
Myhre, E., K. Rasmussen, and A. Anderson, "Serum Lactic Dehydrogenase Activity in Patients with Prosthetic Heart Valves: A Parameter of Intravascular Hemolysis," *Am. Heart J.*, **30,** 463 (1970).
Myhre, E., A. J. Hellum, H. Stormorken, and R. Rootwelt, "Intravascular Hemolysis, Platelet Consumption and Platelet Adhesiveness in Patients with Prosthetic Heart Valves," *Scand. J. Thoracic Cardiovasc. Surg.*, **5,** 13 (1971).
Offeman, R. D., and M. C. Williams, "Shear-Induced Hemolysis: Effects of Blood Chemistry (Including Aging in Storage) and Shearing Surfaces," *Biomat., Med. Dev., Art. Organs,* **4,** 49 (1976).
———, "Material Effects in Shear-Induced Hemolysis," *Biomat., Med. Dev., Art. Organs,* **7,** 359 (1979a).
———, "Observations on Shear-Induced Hemolysis," *Biomat., Med. Dev., Art. Org.*, **7,** 393 (1979b).
O'Rear, E. A., L. V. McIntire, B. O. Shah, and E. C. Lynch, "Use of Rheological Technique to Evaluate Erythrocytes Membrane Alterations," *J. Rheol.*, **23,** 721 (1972).
Phibbs, R. H., "Orientation and Distribution of Erythrocytes in Blood Flowing through Medium-Sized Arteries," *Hemorheology,* A. L. Copley, ed., Oxford (1968).
Reimers, R. C., S. P., Sutera, and J. H. Joist, "Potentiation by Red Blood Cells of Shear-Induced Platelet Aggregation: Relative Importance of Chemical and Physical Mechanisms," *Blood,* **64,** 1200 (1984).
Richardson, E., "Applications of a Theoretical Model for Hemolysis in Shear Flow," *Biorheology,* **12,** 27 (1974).
Shapiro, S. I., and M. C. Williams, "Hemolysis in Simple Shear Flow," *AIChE J.*, **16,** 575 (1970).
Standefer, J. C., and D. Vanderjagt, "Use of Tetramethylbenzidine in Plasma Hemoglobin Assay," *Clin. Chem.*, **23,** 749 (1977).
Stormorken, H., "Platelets, Thrombosis and Hemolysis," *Federation Proc.*, **30,** 1552 (1971).
Sutera, S. P., and M. H. Mehrjardi, "Deformation and Fragmentation of Human Red Blood Cells in Turbulent Shear Flow," *Biophys. J.*, **15,** 1 (1975).
Tanford, C., *Physical Chemistry of Macromolecules,* Wiley, New York (1961).
Turitto, V. T., and H. J. Weiss, "Red Blood Cells: Their Dual Role in Thrombus Formation," *Science,* **207,** 541 (1980).
Turitto, V. T., A. M. Benis, and E. F. Leonard, "Platelet Diffusion in Flowing Blood," *Ind. Eng. Chem. Fundam.*, **11,** 217 (1972).
Von Rosenberg, D. U., *Methods for the Numerical Solution of Partial Differential Equations,* Farrar & Assoc., Tulsa, OK (1977).
Walters, K., *Rheometry,* Wiley, New York (1975).
Wang, N. L., and K. H. Keller, "Augmented Transport of Extracellular Solutes in Concentrated Erythrocyte Suspensions Under Uniform Shear," *J. Colloid. Interface Sci.*, **103,** 210 (1985).
Wielogorski, J. W., T. J. Davy, and R. J. Regan, "The Influence of Surface Rugosity on Hemolysis Occurring in Tubing," *Biomedical Eng.*, **11,** 91 (1976).

Manuscript received May 24, 1985, and revision received July 23, 1986.

See NAPS document on 04434 for 18 pages of supplementary material. Order from NAPS c/o Microfiche Publications, P.O. Box 3513, Grand Central Station, New York, NY 10163. Remit in advance in U.S. funds only $7.75 for photocopies or $4.00 for microfiche. Outside the U.S. and Canada, add postage of $4.50 for the first 20 pages and $1.00 for each of 10 pages of material thereafter, $1.50 for microfiche postage.