RED BLOOD CELL DAMAGE BY SHEAR STRESS

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ABSTRACT A series of careful studies has been made on blood damge in a rotational viscometer. Specific attention has been focused on the effects of solid surface interaction, centrifugal force, air interface interaction, mixing of sheared and unsheared layers, cell-cell interaction, and viscous heating. The results show that there is a threshold shear stress, 1500 dynes/cm^2, above which extensive cell damage is directly due to shear stress, and the various secondary effects listed above are negligible. By analysis of these results and those of prior workers it is shown that the exposure time–shear stress plane is divided into two distinct regimes. In the regime of relatively low stresses and exposure times there is relatively little damage, and the damage is dominated by solid surface interaction effects. In the other regime, at high stresses and exposure times, stress effects alone dominate and very high rates of hemolysis occur. The experimental findings of all prior workers are shown to be consistent when interpreted in this way.

INTRODUCTION

Significant hemolysis is observed in certain patients who have artificial valves or other cardiovascular prostheses (Marsh, 1964; Sayed et al., 1961). Physical forces, particularly shear stress, are believed to be an important causative factor of the hemolysis (Nevaril et al., 1968, 1969). The effects of shear stress on erythrocytes in vitro have been evaluated by several investigators by use of a concentric cylinder viscometer (Nevaril et al., 1968, 1969; Knapp and Yarborough, 1969; Shapiro and Williams, 1970; Steinbach, 1970). Certain of these studies have indicated that shear stress can result in hemolysis and fragmentation of erythrocytes similar to that observed clinically. It has also been shown that in regurgitant aortic prosthetic valves shear stresses can develop which are of the same order as those which cause hemolysis and fragmentation in vitro (Nevaril et al., 1968, 1969).

In a concentric cylinder viscometer, blood is exposed to a known, uniform shear rate and shear stress. Ideally, the effects of shear stress can be evaluated independently of other factors associated with blood flow. Even in this relatively simple system, however, a number of factors other than shear stress may be responsible for erythro-

cyte damage. These factors include: (*a*) interaction of erythrocytes with solid surfaces, (*b*) centrifugal force, (*c*) damage at the air-blood interface, (*d*) cell-cell interaction, and (*e*) viscous heating. In this paper we describe experiments to determine the effects of these factors on erythrocytes in the concentric cylinder viscometer and explore in greater depth the hemolysis of erythrocytes exposed to shear stress.

Solid Surface Interaction

It is well known that the interaction with solid surfaces causes damage to erythrocytes (Blackshear, 1971). Shear stress is transmitted through the fluid itself and presumably has an effect independent of solid surfaces. On the other hand, shearing motion promotes cell rotation and migration, and hence promotes solid surface interaction. We explored this phenomenon in the concentric cylinder viscometer by varying the surface-to-volume ratio at predetermined shear stresses.

Centrifugal Forces

Centrifugal forces generated in concentric cylinder viscometers tend to cause migration of erythrocytes to the outer surfaces. This could result in cell damage independent of the effect of shear stress. On the other hand, the shearing motion tends to cause counter-migration. We studied the net effect experimentally by varying the density and viscosity of the plasma.

Hemolysis at the Air-Blood Interface and Mixing

The concentric cylinder viscometer is designed with a narrow annular gap filled with blood subjected to the desired shear stress. Above the gap there is a layer which is exposed to considerably lower shear stresses. In addition, this upper layer of blood has an interface with air. Because of the lower shear stresses and the air-blood interface, the degree of hemolysis in the upper layer presumably is different from that in the gap. In fact, in prior studies hemolysis in the upper layer has been assumed to be negligible. Mixing of blood in the upper layer with that in the gap during application of shear stress may distort results of studies aimed at establishing levels of hemolysis. We determined both the magnitude of hemolysis in each region and the effect of mixing on interpretation of results.

Cell-Cell Interaction

Studies were conducted to determine whether shear stress acts primarily on individual cells or through cell-cell interaction in a shear field. This question was evaluated by measuring hemolysis of erythrocytes at fixed shear stresses but with concentrations of erythrocytes varying from 0.30 to 60%.

Viscous Heating

At high shear rates used in some viscometric studies the rise in temperature due to viscous heating may be important as a cause of hemolysis. We have studied this effect in a direct way.

APPARATUS AND LABORATORY METHODS

For these studies, we modified a Fann Model 38A viscometer (Fann Instrument Corp., Houston, Tex.) by retaining the stand and torque readout head but redesigning the motor, drive, controller, torque shaft, and cylinders to yield higher shear rates and stresses. The basic configuration of the gap is cone-and-plate at the bottom and concentric cylinder along the sides. It is designed so that fluid in the entire gap is subjected to the same shear stress. Two different cup-and-bob sets were used. One had a gap width of 0.010 cm (configuration I) and the other 0.038 cm (configuration II). The cups are connected to the drive shaft of the viscometer by an adjustable collar allowing one to set the bottom gap and to adjust the concentricity of the cup and bob. A summary of physical characteristics appears in Table I. More complete descriptions of the apparatus and methods have been given (Nevaril et al., 1968, 1969; Leverett, 1970).

Blood was collected in acid-citrate-dextrose (ACD), National Institutes of Health Formula A, in plastic bags from healthy, fasting, human male subjects. Studies were completed within 24 hr after phlebotomy. Before use, the blood was allowed to come to room temperature, 21°C. All surfaces to which blood was exposed were siliconized using Clay-Adams Siliclad (Clay-Adams, Inc., Parsipany, N. J.). In each study, blood was exposed to the desired shear stress in the viscometer for 2 min. Unless otherwise stated, the gap width was 0.010 cm (configuration I, Table I).

The procedure for conducting an experiment was to add 6.5 ml of blood to the cup, carefully assemble the viscometer to exclude air bubbles, accelerate the cup until the desired torque was reached, maintain the desired shear stress for 2 min, and then stop the viscometer. The time involved in acceleration and deceleration was about 20 sec. Samples of blood were withdrawn through a valve in the cone and plate section. The first drop was discarded and the next eight drops, 0.4 ml, were used to determine per cent hemolysis. Total plasma hemoglobin of the sample was measured by the benzidine method (Hanks et al., 1960). Total hemoglobin

TABLE I
PHYSICAL CHARACTERISTICS OF PLATENS

Platen	O.D.	I.D.	Mass	Length
	cm	*cm*	*g*	*cm*
Outer cylinder	4.561	3.522	126.1	6.30
Inner cylinder I	3.502		115.1	4.47
Inner cylinder II	3.446		71.8	1.81

Platen assembly	Gap	Shearing rate	Gap volume	Total volume	Surface/ volume
	cm	*sec^{-1}/rpm*	*ml*	*ml*	*cm^{-1}*
Configuration I	0.010	18.21	0.47	6.50	210.8
Configuration II	0.038	6.49	1.29	7.25	78.5

was measured as cyanmethemoglobin. Hematocrit was determined with a capillary tube centrifuge.

In studies of centrifugal effects, human albumin (crystalline, B grade, Calbiochem, San Diego, Calif.) was dissolved in the autologous plasma to a concentration of 34% by weight. The erythrocytes were then resuspended in the albumin-containing plasma. The cell density distribution compared with plasma was determined by erythrocyte count of upper, middle, and lower fractions after centrifugation.

In designing our studies of the degree of mixing, we were uncertain whether red cells and plasma would be distributed in the same manner. Hence, two radioactive tracers were used. The erythrocytes were labeled with radiochromium (^{51}Cr), and the plasma was labeled with radioiodinated (^{125}I) human serum albumin (Cooper and Owen, 1956). The viscometer was filled with 5 ml of unlabeled blood. Then 0.5 ml of the doubly labeled blood was slowly added to the upper layer, followed by 1.0 ml of unlabeled blood. This blood was subjected to shear stress in the viscometer and then drained in small aliquots. The radioactivity of each aliquot was determined by counting samples in a well-type scintillation counter. Changes in hematocrit were made by adding or subtracting known volumes of autologous plasma from samples with known volumes and hematocrits.

Measurements of temperature changes were made using silicone oil, 5 centistoke Dow Corning No. 200 (Dow Corning Corp., Midland, Mich.), which has a viscosity approximately the same as blood. The known viscosity-temperature characteristics of this fluid give a direct measurement of the average temperature of the fluid.

The work discussed here is all from two different configurations of a single rotational viscometer; however, it is interesting to point out that in this laboratory we have developed three different rotational viscometers of considerably different design since the experimental program started in 1964, and these instruments give remarkably consistent results. Ordinary viscometers cannot operate in the shear range employed in this work so special equipment is necessary. We have also studied capillary viscometric results reported by other workers and in the last section of this paper we show how these results are related to those in other viscometers.

RESULTS

Solid Surface Interaction

In configuration I the viscometer had a gap width of 0.010 cm and surface-to-volume ratio of 210.8 cm^{-1}. The gap width in configuration II was 0.038 cm with a surface-to-volume ratio of 78.5 cm^{-1}. Hence, we conducted a series of experiments with two configurations identical as far as possible except for the change in surface-to-volume ratio by a factor of 2.68.

In studies of blood of two subjects at several shear stresses, hemolysis using the two configurations did not differ significantly (Fig. 1). Hemolysis, thus, seems independent of the surface-to-volume ratio and, therefore, we believe interaction of erythrocytes with the surface is unlikely as an explanation of hemolysis under the conditions of the experiments.

Centrifugal Forces

The density of human erythrocytes varies between 1.085 and 1.115 g/cm^3, whereas plasma density is approximately 1.030 g/cm^3. Hence at the relatively high rates of

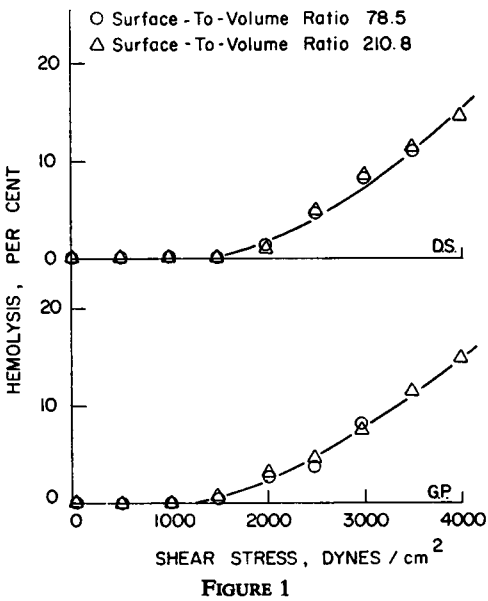

FIGURE 1

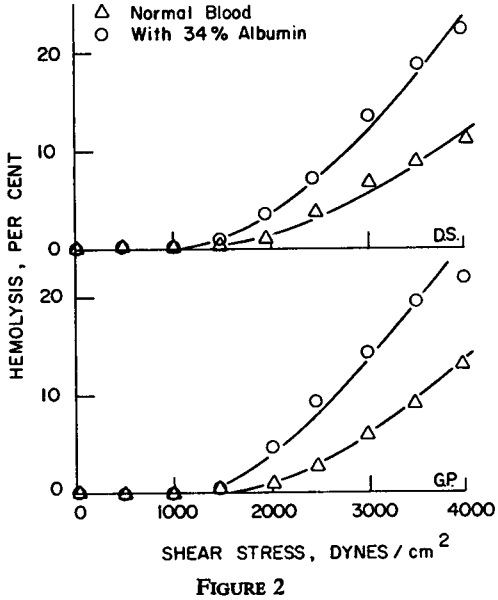

FIGURE 2

FIGURE 1 Hemolysis resulting from shear stress in two different configurations with different surface-to-volume ratios.

FIGURE 2 The effect of change in density and viscosity on hemolysis.

rotation used in our studies, there is an appreciable tendency for cells to migrate to the outer surface. At the highest rotational speeds used, the relative centrifugal force approximates 600 *g*. Surface interaction related to centrifugal forces is not likely to be important as a cause of hemolysis because we found no effect of surface in the studies presented above (Fig. 1); however, we decided to investigate this further by making the erythrocytes approximately neutrally buoyant by the addition of albumin to the plasma. Erythrocytes suspended in plasma containing 34% human serum albumin were subjected to shear stresses of 500–4000 dynes/cm^2. Under these conditions centrifugal forces are greatly reduced because of the reduction in difference in density between cells and plasma, and because of a threefold increase in viscosity which permits lower rotational rates for comparable shear stresses.

Because there is a range of densities in the erythrocytes, it was not possible to make them all neutrally buoyant. The density distribution of the cells relative to the plasma expressed as per cent neutral, more dense, and less dense, respectively, for the two subjects were G.P. (10, 80, and 10%), and D.S. (10, 50, and 40%). The threshold shear stress for hemolysis was unaffected by addition of the albumin (Fig. 2). At higher shear stresses erythrocytes suspended in 34% albumin were slightly more susceptible to hemolysis than cells suspended in normal plasma.

This increase in fragility of the cells with the very high concentration of albumin was not totally unexpected since at concentrations of 34% and higher, we observed minor changes in morphology of the cells. It should be emphasized however, that

the increased fragility is slight and constitutes a change in the opposite direction to that expected if centrifugal effects were important. That is, if the centrifugal effects were important as a cause of hemolysis, the addition of albumin should have diminished hemolysis.

This experiment was also conducted using configuration II of the viscometer. The results were very similar to those obtained with configuration I. Hence, it appears the centrifugal effect has little importance under the conditions of our experiments.

Air Interface Hemolysis and Mixing Studies

When using a concentric cylinder viscometer, fluid in the layer above the gap is subjected to low shear stresses while that in the gap is exposed to relatively high shear stresses. This upper layer separates the blood in the gap from the air interface. In our studies the volume of the upper layer was 6.0 ml compared to volumes in the gap of 0.474 ml and 1.12 ml for configurations I and II, respectively. The air interfacial area was 3.4 cm^2. Mixing between the fluid layers must be considered in the interpretation of results. In addition, hemolysis may occur in the upper layer, at the blood-air interface.

To evaluate hemolysis of blood in the upper layer compared with that in the gap, we subjected blood to shear stress and then drained the viscometer from the bottom in aliquots corresponding to the volumes of the gap and the upper layer. The first 0.5 ml of effluent was regarded as coming from the gap. The next 1.0 ml was considered mixed and discarded. The remainder of the blood represented that in the upper layer. Values of plasma hemoglobin of blood in the gap were several times greater than those of blood in the upper layer (Fig. 3). Because the volume of blood in the upper layer is considerably greater than that in the gap, the total amount of

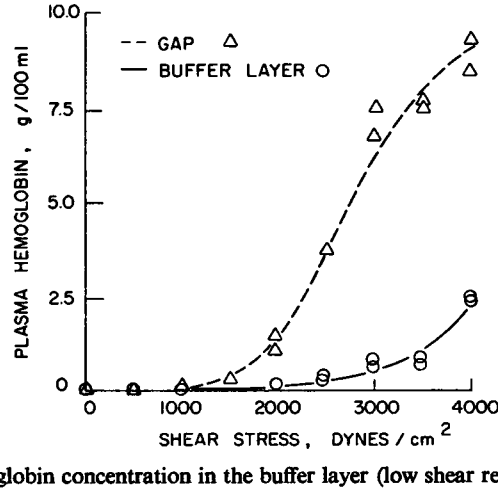

FIGURE 3 Hemoglobin concentration in the buffer layer (low shear region) compared with the concentration in the gap (high shear region).

plasma hemoglobin generated by hemolysis was approximately equal in the two regions.

The extent of mixing was determined by adding isotopically labeled plasma and erythrocytes to the upper layer with unlabeled blood in the gap and then subjecting the sample to shear stress. The radioactivity of the blood in the gap was then measured (Fig. 4). With application of a shear stress of 1000 dynes/cm^2, the tracer concentration in the gap is about 40% of the over-all average concentration. It approaches 60% with shear stress of 4000 dynes/cm^2. If there were no mixing the value would be 0% and if mixing were complete, it would be 100%.

The point near zero shown in Fig. 4 represents a study made with application of shear stress of 8 dynes/cm^2. No evidence of mixing was found. Thus, the procedures of filling and draining the viscometer do not result in significant mixing of blood in the upper layer with that in the gap. The similarity of the results with ^{125}I-labeled plasma and those with ^{51}Cr-labeled erythrocytes indicates that the degree of mixing of plasma and erythrocytes in the upper layer with blood in the gap is similar.

The problems of mixing and hemolysis at the air-blood interface can be subjected to mathematical analysis. Consider a simple two-chamber model of the process. One chamber, 1, represents the gap of the viscometer and the other chamber, 2, represents the upper or buffer layer. If each chamber is regarded as being completely mixed, then material balances on the material being transported (tracer in the tracer experiments or hemoglobin in the ordinary blood runs) yields the pair of differential equations

$$\frac{dC_1}{dt} = \frac{q}{V_1}(C_2 - C_1) + \frac{k_1}{V_1}, \qquad (1)$$

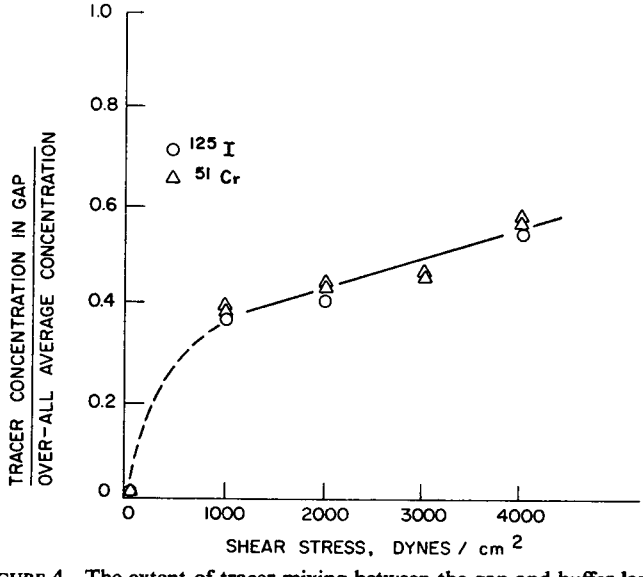

FIGURE 4 The extent of tracer mixing between the gap and buffer layer.

$$\frac{dC_2}{dt} = \frac{q}{V_2}(C_1 - C_2) + \frac{k_2}{V_2}, \tag{2}$$

where C_1 and C_2 denote concentrations in the two chambers, V_1 and V_2 the volumes of the two chambers, k_1 and k_2 denote over-all hemolysis rates in the two chambers, and q denotes the mixing rate, or rate of flow between the two chambers.

The solution to the system of equations may be obtained by elementary means. The solution is given below for initial concentrations $C_1(0)$ and $C_2(0)$.

$$C_1 = \frac{C_1(0)V_1 + C_2(0)V_2}{V_1 + V_2} + \left(\frac{C_1(0)V_2 - C_2(0)V_2}{V_1 + V_2}\right)\exp\left[-qt\left(\frac{V_1 + V_2}{V_1V_2}\right)\right] + \frac{V_2}{q}\left[\frac{k_1V_2 - k_2V_1}{(V_1 + V_2)}\right]\left\{1 - \exp\left[-qt\left(\frac{V_1 + V_2}{V_1V_2}\right)\right]\right\} + \frac{k_1 + k_2}{V_1 + V_2}t. \tag{3}$$

The solution for C_2 may be obtained by interchanging the subscripts 1 and 2 in equation 3.

The mixing and air hemolysis problem was attacked in the following sequence:

(*a*) The tracer experiments were used to determine q as a function of shear rate or shear stress. All other quantities in the equation are measured or known in the tracer experiments because k_1, k_2, and $C_1(0)$ are zero.

(*b*) Using these determined values of q, it is possible to analyze the hemolysis experiments. Using the known values of q, the hemoglobin concentration measurements may be used to determine k_1 and k_2, the hemolysis rates of the two regions, as functions of shear stress.

(*c*) Finally, with all parameters in the equations known, it is possible to calculate a correction factor from which one may infer the magnitude of the error incurred from overlooking the effect of interface hemolysis and mixing.

The results of q, the mixing rate parameter, are given in Table II. As expected, q is a monotonically increasing function of shear rate or shear stress. These runs were made on a blood of viscosity of 3.63 centipoise.

Mixing rates for the ^{51}Cr-labeled cells and the ^{125}I-labeled plasma are not significantly different (Table II). Further, the maximum mixing flow would not ac-

TABLE II

VALUES OF THE MIXING RATE PARAMETER

Shear stress	q based on ^{51}Cr	q based on ^{125}I
dynes/cm²	*ml/min*	*ml/min*
8	0.200×10^{-5}	0.202×10^{-5}
1000	0.120	0.106
2000	0.133	0.120
3000	0.131	—
4000	0.196	0.180

count for the free hemoglobin levels in the low shear region because less than one volume turnover occurs during the normal 2 min experiment. Hence, there is appreciable hemolysis in the low shear, upper region.

The values of q in Table II were used with the initial conditions of zero concentration of free hemoglobin to determine values of the hemolysis rates, k_1 and k_2. These results, calculated from the measurements of the type shown in Fig. 3, are given in Table III.

This table clearly show that significant hemolysis does take place in the buffer region (subscript 2) and it presumably occurs primarily at the blood-air interface since our studies indicate that other effects should be small at the low shear rate in the upper layer. The over-all hemolysis rate in the upper layer actually becomes larger than that in the gap at the highest shearing stress shown, although, of course, it is less per unit volume. The hemolysis rate in the upper layer is much less than that in the gap in the threshold region, 1000–2000 dynes/cm^2.

Finally, it is possible to calculate a correction to the observed values of hemoglobin release in the experiments. The product k_1t gives our improved estimate of the total hemolysis in the gap for a given run in the viscometer. The ratio of this product to the measured hemoglobin level (or its equivalent calculated from the system of equations) is the correction factor f or error estimate shown in Table III for a run time of 2 min.

The observed hemolysis levels which were reported in Figs. 1–3 could be divided by the correction factor f of Table III to yield the improved estimate of the actual hemolysis level. It is satisfying to note that the correction does not exceed 20% even at the extremely high stress of 4000 dynes/cm^2 and is 10% or less in the range 1000–2000 dynes/cm^2.

Cell-Cell Interaction

Prior workers (Keshaviah, 1970) have suggested that the mechanism of erythrocyte damage involves interaction between cells. To test this idea we studied the effect of shear stress of 3000 dynes/cm^2 on blood specimens with hematocrits varying

TABLE III
HEMOLYSIS RATES IN GAP AND BUFFER LAYER WITH CORRECTION FACTOR

Shear stress	k_1	k_2	Correction factor, f
dynes/cm²	*mg/min*	*mg/min*	
8	0.1	0.01	1.00
1000	0.3	0.01	0.98
2000	2.7	0.7	0.91
3000	21.2	15.2	0.88
4000	26.7	63.9	0.80

between 0.3 and 60%. Because collision frequency is a function of the concentration of erythrocytes, hemolysis should increase as hematocrit increases if the cell-cell interaction hypothesis of cell destruction is correct (Shapiro and Williams, 1970). We found that varying the hematocrit did not change the percentage of red cells hemolyzed (Fig. 5). We have concluded that cell-cell interaction is not an important mechanism of normal erythrocyte destruction in the concentric cylinder viscometer.

Viscous Heating

Viscous heating is potentially a problem when studying the effects of shear stress on erythrocytes in a concentric cylinder viscometer. The gap temperature at steady state for various shear stresses is shown in Fig. 5. Substantial increases in temperature may be observed. At a shear rate of 10^5 sec^{-1} (corresponding to a shear stress of about 3000 dynes/cm^2) the steady-state temperature rise is approximately 20°C. Depending on the initial temperature, this rise in temperature could be sufficient to cause red cell damage.

Fig. 6 also shows the temperature rise for the 2 min time period used in the blood damage studies. For the case mentioned above in which the steady-state temperature rise is about 20°C, the 2 min temperature rise is about 10°C. Because the initial temperature of blood in our studies was 21°C, a rise of 10°C would not raise the temperature to a level which would cause hemolysis. Prior studies have shown that a temperature of about 49°C will cause significant hemolysis and fragmentation.

There are circumstances, however, in which the heating may be a very important problem in viscometric studies. We have analyzed the heating problem to develop the information needed to estimate the temperature rise. Such estimates are needed in planning experiments and in assessing or designing new viscometric equipment. Details of the study are available elsewhere (Leverett, 1970), and the results are only briefly summarized here. Blood and both components of the concentric cylinders may be assumed to be at a single, uniform temperature without significant error.

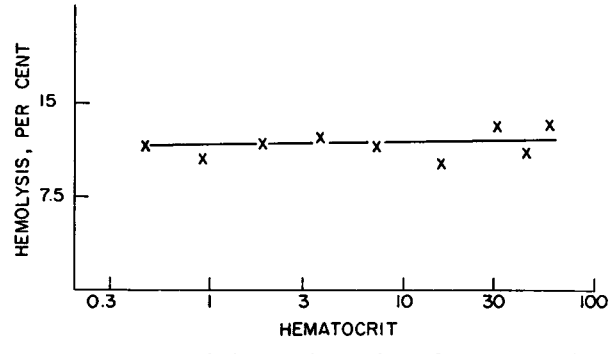

FIGURE 5 Hemolysis at various values of the hematocrit.

Assuming the components are metal, the conductivity is high compared to the relatively low air interface heat transfer coefficient. The air side heat transfer resistance completely dominates the process for practical purposes. There have been no previous measurements of heat transfer coefficients from vertical rotating cylinders. Because this heat transfer coefficient is of primary importance in determining the temperature rise, we have made the necessary measurements and calculations (Fig. 7). The cylinder diameter is the length variable and the velocity variable is the diameter–angular velocity product. The results should apply to other cylinders of different diameters under laboratory conditions because the physical properties of air do not change greatly with temperature over the relatively narrow

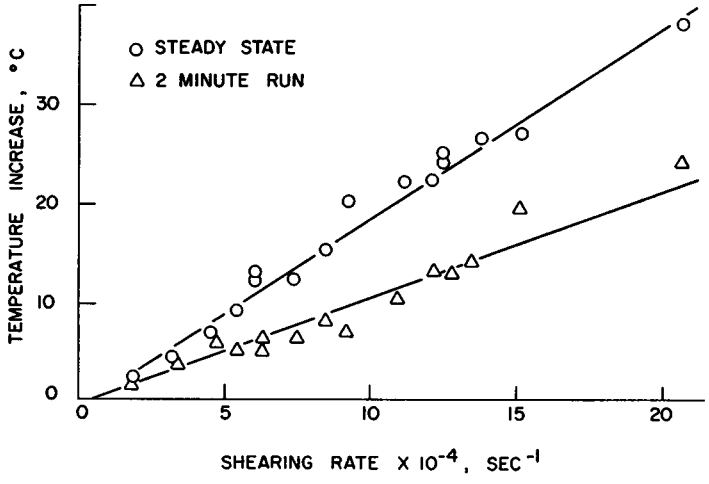

FIGURE 6 Temperature increase due to viscous heating.

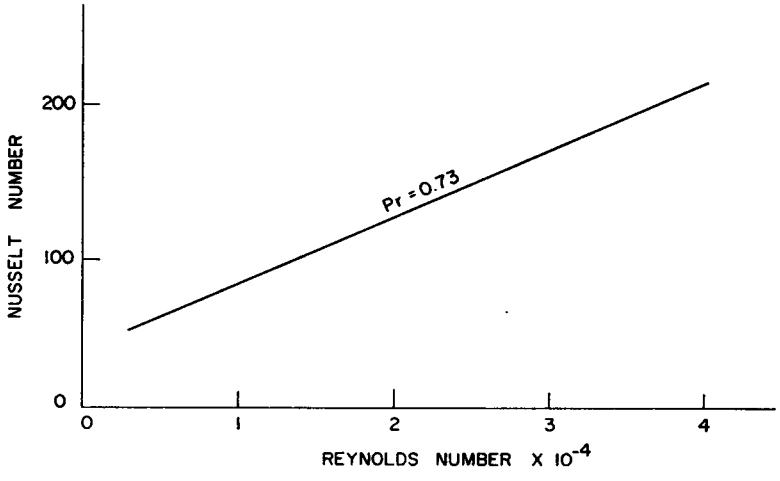

FIGURE 7 Heat transfer from a rotating vertical cylinder.

range of temperatures involved. The results were calculated based on thermal conductivity of air of 6.0×10^{-5} cal cm^{-1} sec^{-1} $°K^{-1}$ and a kinematic viscosity of 0.151 cm^2 sec^{-1}.

Using the definition of the heat transfer coefficient and the expression for viscous heating, we developed an expression for the temperature rise applicable to any concentric cylinder viscometer. Using the assumptions indicated above, the transient energy balance reduces to a simple ordinary differential equation which may be solved for the temperature increase with time.

$$\frac{dT}{dt} = \frac{hA}{\sum m_i Cp_i} (T - T_0) + \frac{V\mu\dot{\gamma}^2}{\sum m_i Cp_i}, \quad (4)$$

where $V\mu\dot{\gamma}^2$ is the rate of heat generation (see Bird et al., 1965, for example), $\sum m_i Cp_i$ is the total heat capacity, A is the area for heat transfer, h is the heat transfer coefficient, and μ is the viscosity. We have also solved a slightly more complicated form in which the temperature dependence of viscosity is taken into account (Leverett, 1970).

The heat transfer coefficient was determined from runs at steady state. Then equation 4 was used to predict the temperature as a function of time. The calculated temperature rise from the equation agrees with the measurements to within 0.5°C. Hence, these results may be used with confidence to analyze the heating problem in the design of new viscometric equipment and in planning experiments.

DISCUSSION

The study is apparently the first on blood damage in which the various secondary effects in the rotational viscometer have been studied in a systematic way. We are able, therefore, to cast light on several points which have been somewhat controversial in the past.

Primary Results

The most important finding in this work is that the threshold level for extensive erythrocyte damage directly due to shear stress is about 1500 dynes/cm^2. Careful study of secondary effects has revealed that no serious error is incurred from these sources in properly planned experiments. At shearing stresses near and above the threshold level, the cell damage is due to shear stress acting directly on the cells. It should be emphasized that neither cell–solid surface interaction nor cell-cell interaction appear to play an important role in this high stress regime. The shear stress effect alone dominates. These results confirm those of Nevaril et al. (1968, 1969) from this laboratory. Nevaril did not vary surface-to-volume ratio but he did vary the viscosity of the suspending medium. By this means he was able to vary the shear rate for given values of the shear stress, and he found no independent effect of shear rate.

It should be pointed out that several prior workers (Bernstein et al., 1967; Knapp and Yarborough, 1969; Shapiro and Williams, 1970; Steinbach, 1970) have stated that surface effects are of primary importance in seemingly similar studies. The reason for the apparent contradiction lies in the fact that all other workers have used a maximum shear stress about one order of magnitude lower than those used in this work. Ordinary viscometric equipment is not designed for the high stresses used in this work. Notice in Fig. 1 that the threshold, for shear stress damage to become important, is in the neighborhood of 1500 dynes/cm^2. Prior workers using shear stresses of a few hundred dynes per square centimeter or lower saw little damage by shear stress mechanism; however, even at very low stresses, if the viscometer is run for longer periods of time, measurable amounts of hemolysis will occur. In this low stress regime used by prior workers, it is clear that the solid surface interaction is of paramount importance. We have confirmed the findings of other workers that for such relatively low stresses the cell damage is almost directly proportional to the surface-to-volume ratio in the gap.

We have also shown that substantial amounts of hemolysis may occur at the air interface and that significant mixing occurs between the regions of the viscometer. Our analysis shows that for the conditions of our experiments the error from these sources is not large; however, all prior workers using rotational viscometers have operated at much lower rates of hemolysis (much lower stresses) in the gap. Therefore, it seems likely in these prior studies that the air interface and mixing contributions are a much higher fraction of the total hemolysis and may in some cases mask the phenomena under study.

The heating studies show that the temperature rise was not excessive in our experiments. We have also developed design procedures for predicting the temperature rise in any rotational viscometric equipment. Similarly the centrifugal force effect appears to be negligible under the conditions of our experiments.

The studies on cell-cell interaction indicate that this effect is unimportant, contrary to the suggestion of some prior workers. It appears that in the high stress regime, the stress field interacts directly with the individual erythrocytes. Previous studies on the effect of hematocrit (Shapiro and Williams, 1970) have yielded similar conclusions but have been based on a relatively narrow range of variation in hematocrit.

Prior Work

All prior workers using rotational viscometers have operated in the low stress regime well below the threshold for direct stress damage.

Several workers have investigated the high stress regime using equipment other than the concentric cylinder viscometer. Rooney (1970) used a pulsating gas bubble immersed in the blood, and Williams et al. (1970) carried out a similar study using an oscillating wire. In both cases the authors analyzed the flow, and they were able

to estimate the maximum shear stress in the flow field. They found a threshold stress for damage of 5600 dynes/cm^2 for the bubbles. Only a small fraction of the specimen is exposed to the maximum stress at any one time in these oscillating flows. Hence, the observed threshold should be expected to be higher than that found for the uniform stress field of the present work.

Forstrom (1969) and Blackshear (1971) used jets of blood and jets of other liquids into blood to study the effect of shear stress. The jet device has the important disadvantage that there is no completely satisfactory way of characterizing the shear stress. The stress depends strongly on position and there is no well defined maximum stress. Near the entrance, in the high stress region of most interest, the stress varies approximately as $1/x^2$ where x is distance from the entrance (Schlichting, 1968). Using a stress based on an average inlet velocity gradient, the authors reported a threshold stress of 40,000 dynes/cm^2. There is no direct way of comparing this figure with those from other devices in which the maximum stress is known; however, the calculated stress presumably is a valid order of magnitude estimate. The higher threshold is in the direction one would expect because the cells are subjected to stress for very short periods of time in the jet.

Keshaviah (1970) and Blackshear (1971) studied hemolysis in canine blood flow through capillaries and reported results in terms of velocity. The maximum shear stress can be calculated for these flows. The resulting threshold values are about 4500 dynes/cm^2 for ordinary capillaries and about 7000 dynes/cm^2 for capillaries with a smooth, tapered entrance.

Bacher and Williams (1970) used capillary tubes in studies on bovine blood. There may be some difficulty in comparing this work with others since the authors stated that the age of the blood made it more susceptible to damage than fresh blood. This work shows a threshold for damage of about 5000 dynes/cm^2. The authors give some arguments in favor of a cell–solid surface mechanism although their data is not sufficient to give clear support to arguments for any mechanism.

The Effect of Exposure Time

The time of exposure to shearing stress is an important variable which has received inadequate attention in the past. We have collected and analyzed all prior work on hemolysis with attention focused on the exposure time.

Exposure time is measured directly in the concentric cylinder viscometer. In other equipment estimates of the time are more difficult, but in even the most complex flows it is possible to make an order of magnitude estimate. For the oscillating wires and bubbles the exposure time was taken to be the boundary layer length divided by the product of the maximum shear rate and the boundary layer thickness. For the jet experiments the exposure time was based on the time required for the stress on a cell to decay by one order of magnitude, based on a simple analysis of the turbulent jet (Schlichting, 1968).

A summary of the state of studies on red blood cell damage is given in Table IV. This table brings all the results together in a way which makes it easy to see why there have been numerous disagreements among various workers as to the mechanism and threshold levels for damage. Exposure time and stress level are the two primary parameters. In the high stress region the threshold level may be seen to vary in a monotonic way with exposure time. At stresses below 1500 dynes/cm^2 there is little direct damage due to shear stress. Solid surface interaction and other shear rate–related phenomena (such as air interface interaction) predominate in the low stress regime and the levels of hemolysis per unit time are relatively low. Hence, it appears that the primary reason for disagreements among prior workers is that different workers have studied different regimes of the exposure time–stress domain. Most prior studies have been insufficiently detailed to establish mechanisms for damage.

The results are presented in Fig. 8 in which the division of the shear stress–time domain into two distinct regimes is displayed. The dotted line represents our estimate of the threshold for extensive shear stress damage. Considering the diverse flows and different bloods and conditions used by the various workers, the results are remarkably consistent. The envelope designated "prior workers" pertains to

TABLE IV
SUMMARY OF EFFECT OF SHEAR STRESS ON HEMOLYSIS

Type of exposure	Order of magnitude of exposure time	Threshold level of damage	References and comments
	sec	*dynes/cm²*	
Turbulent jet	10^{-5}	40,000	Forstrom (1969) and Blackshear (1971)
Oscillating wire	10^{-4}	5600	Williams et al. (1970) (human and canine)
Oscillating bubble	10^{-3}	4500	Rooney (1970) (human and canine)
Capillary flow	10^{-2}	5000	Bacher and Williams (1970) (bovine blood)
Capillary flow	10^{-2}	4500–7000	Keshaviah (1970) and Blackshear (1971) (canine blood)
Concentric cylinder	10^{2}	1500	This work
Concentric cylinder, maximum stress, 600 dynes/cm²	10^{2}–10^{3}	Relatively little hemolysis per unit time	Shapiro and Williams (1970) (surface effects dominate)
Concentric cylinder, maximum stress, 250 dynes/cm²	10^{3}	Relatively little hemolysis per unit time	Knapp and Yarborough (1969) (surface effects dominate)
Concentric cylinder, maximum stress, 600 dynes/cm²	10^{3}	Relatively little hemolysis per unit time	Steinbach (1970) and Blackshear (1971) (surface effects dominate)

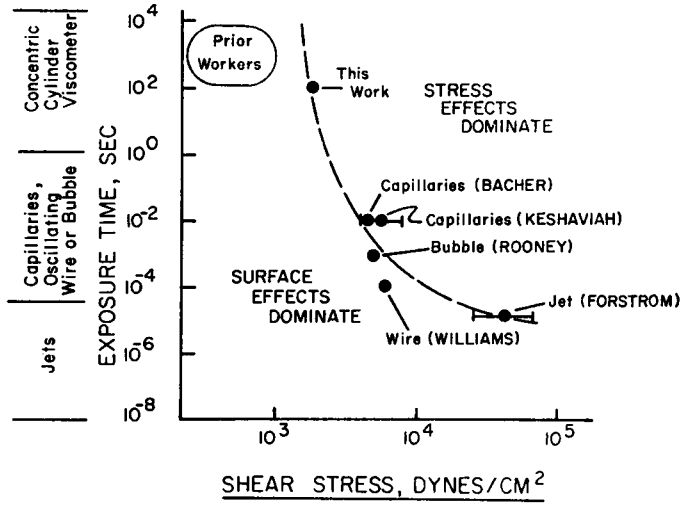

FIGURE 8 The effect of exposure time on the threshold shear stress.

prior workers using the concentric cylinder viscometer. As indicated earlier, all this prior work was at stresses below the threshold for extensive damage. The times and the stresses are, of course, only order of magnitude estimates for the more complicated flows. Even this accuracy, however, is more than has been previously available, and this accuracy is adequate for many applications in the design and analysis of prostheses.

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