RED BLOOD CELL DAMAGE BY
SHEARING STRESS - A STUDY ON SECONDARY
EFFECTS IN THE CONCENTRIC CYLINDER VISCOMETER

by

L. Byron Leverett

A Thesis Submitted in Partial Fulfillment of
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Thesis Director's Signature

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ABSTRACT

RED BLOOD CELL DAMAGE BY SHEARING STRESS - A STUDY ON SECONDARY EFFECTS IN THE CONCENTRIC CYLINDER VISCOMETER

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L. BYRON LEVERETT

The causes of red cell damage in artificial prostheses have been studied by several investigators. The effects of shearing stresses on cells have been investigated in the concentric cylinder viscometer. It has been demonstrated that at high shearing rates, such as can occur in an insufficient artificial valve, red cells undergo hemolysis and morphological changes similar to those seen in vivo.

There are difficulties involved in the interpretation of studies of blood trauma. The difficulties arise through the possibilities of trauma from secondary effects in addition to the damage caused by shear stress. These secondary effects were studied experimentally in a concentric cylinder viscometer.

One important effect is that associated with material interaction. It is well known that cell damage can result from interaction with solid surfaces. Therefore, there is a problem distinguishing damage from surface effects as opposed to damage from any other source. The surface interaction was investigated by varying
the surface to volume ratio of the concentric cylinder viscometer by a factor of 2.68. It was found that at high shear stresses, above the critical shearing stress, no effect could be observed over the range of shear stresses investigated.

Another secondary effect investigated was that of centrifugal forces. This was done by adding serum albumin to the blood to vary the relative density of the plasma to the cells. This addition of albumin increases the viscosity. Therefore, blood can be subjected to the same shear stresses at lower centrifugal fields. It was found that the centrifugal field had little effect on hemolysis and that in the situation with some of the cells being neutrally buoyant there was a slight augmentation of hemolysis rates.

In some concentric cylinder viscometers used for blood studied a buffer layer is provided to separate the blood undergoing shear from a gas interface. There exists the possibility of mixing between the blood undergoing shear and that in the buffer layer. This was investigated using a dual isotope tag. It was found that mixing does occur. A model is provided to account for the mixing and to calculate the true hemolysis rates. It was also found that at high rotational rates hemolysis does occur in the buffer layer.

As blood is sensitive to thermal damage the heat dissipation
characteristics of the concentric cylinder viscometer were investigated. Design curves were obtained for steady state and for transient operation.
ACKNOWLEDGEMENTS

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<td>$\phi$</td>
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INTRODUCTION

With the ever increasing interest in prosthetic devices in the circulatory system, there has been an increased study of the effects of shear interaction with red blood cells. The crux of this interest is the understanding of the situations that damage red blood cells in the artificial implants.

The concentric cylinder viscometer has been applied to the investigation of shear interaction with the red blood cell. Unfortunately, some of the physical characteristics of the concentric cylinder viscometer relative to the delineation of the traumatic effects have not been properly characterized, so that existing data cannot be properly interpreted and future studies designed with greater facility.

The specific areas considered in this study are those of (1) viscous heating, (2) centrifugal sedimentation of red blood cells in the annular shear gap, (3) the influence of surface effects on the hemolysis rate during shear, and (4) the effect of the mixing of the sheared and unsheared blood.
BACKGROUND

Most of the experiments discussed in this work were run with blood. As an aid to understanding, the following simplified description of blood is provided.

Blood is composed basically of two phases: the plasma (the suspending liquid) and the cells (the suspended particles). The plasma is about 90% water by weight, 7% plasma protein (mainly albumin), 1% inorganic, and the remainder various organic substances. Plasma has a specific gravity of approximately 1.03.

The cells can be separated from the plasma by centrifugation. The percentage of the total volume of the blood occupied by the cells is known as the hematocrit and is normally 45-50%. The cells are of three main types: erythrocytes (red blood cells), thrombocytes (platelets), and leucocytes (white cells). Normally, the red blood cells compose 99% of the cell volume. They are the subject of main interest in this study.

The red blood cell is a biconcave discoid of a diameter on the average of 7.7 microns and has a maximum thickness of 2 microns. The red cell membrane has a complex structure of lipids and proteins that has not been characterized in full. The interior of the red blood cell contains hemoglobin in the concentration of 35 gms
per 100 ml. This high content of hemoglobin is responsible for the higher specific gravity of the red blood cell as compared to plasma, and is approximately 1.10 with a range of 1.085 to 1.115. If the membrane of the cell is damaged the hemoglobin will leak into the plasma, leaving the red cell membrane, which is then termed a "ghost".

For a more complete discussion of blood, one should refer to one of the many good texts (24, 44) given in the bibliography of this paper.
LITERATURE SURVEY

From early work with cardiovascular prostheses it became evident that hemolysis could be of great concern. This knowledge resulted in efforts to study the various physical effects which might be responsible for red blood cell destruction.

In particular, several investigations considered the effects associated with the flow in a concentric cylinder viscometer. An indirect, early related attempt toward this was the work by Fleish and Fleish$^{(16)}$ with the hemoresistometer. This is not a concentric cylinder viscometer, but rather a cube rotating inside of a cup. They did find hemolysis but it is very difficult to evaluate what mechanism might be associated with this. More recently, the works of Blackshear, et al$^{(7)}$, Knapp and Yarborough$^{(22)}$, and Shapiro and Williams$^{(3)}$ with concentric cylinder viscometers also found hemolysis. But the interpretation of the results was that the responsible mechanism was associated with wall interaction and the probability of the red cell interaction with the wall. In particular, in the case of Shapiro the maximum shear stress was 1200 dynes per centimeter square and Knapp only 250. Nevaril$^{(30,31)}$ in his measurements discovered that there was a critical or threshold shear stress associated with red cell damage, this being 1500 dynes per centimeter square.
square. The nature of the results strongly indicated that the mode of action was one of shear stress damage rather than of wall interaction, but this was not definitely shown. Bernstein, et al, \(^{(2)}\) also concluded that the hemolysis in a concentric cylinder viscometer was dependent on the surface area and probability of the cells reaching the surface. From jet experiments Berstein found a critical velocity of 1,000 centimeters per second above which hemolysis was quite evident. These results plus results of others were interpreted by Bleustein and Moukros \(^{(8)}\) rather to be associated with damage proportional to the local energy dissipation.

In the interpretation of the various previously reported results many problems have become apparent. Probably the most obvious of these is in association with the fact that blood is not truly Newtonian. This has been clearly pointed out by Merrill, et al \(^{(27, 28, 29)}\), Charm and Karlem \(^{(10)}\), Harris \(^{(17)}\) and others. Further complications arise with the use of the concentric cylinder viscometer on blood. As Danon and Marckousky \(^{(14)}\) clearly demonstrates, the density of red blood cells is not constant. He shows that the age of cells is related to their density and that, therefore, there is a distribution of densities in a population of blood cells. The effect of this density variation on blood cells, plus the fact that the density of
plasma is less than that of red blood cells, is that in the rotational environment evident in the concentric cylinder viscometer, a centrifugal force is applied to the red blood cell. The effect of this would be to cause the red blood cells to migrate toward the outer wall, resulting in a hematocrit variation across the gap. It has been pointed out by Wells and Merrill\(^{42}\) and others that the viscosity is dependent upon hematocrit; therefore, there would be a viscosity variation across the gap.

Another problem in the interpretation of the results is that associated with temperature. Merrill, et al,\(^{(28)}\) found that there is a dependence of viscosity on temperature and fat concentration. Blackshear, et al\(^{(6)}\) discovered that at constant pressure drop, hemolysis rate increases with temperature. Bernstein, et al\(^{(2)}\) on the other hand, discovered that in a concentric cylinder viscometer that over a temperature range of 15 to 37 degrees centigrade the temperature versus hemolysis rate in fasting subjects was very close to constant. This result was not found in non-fasting individuals.

Work has been done by Bird and Turian\(^{(4,36)}\) on the problem of heat generation and the effect of this on the viscosity in a cone and plate viscometer. In these studies they consider specified boundary temperature conditions. This is not the situation in many experimental
studies. The temperature distribution and rise in the concentric cylinder portion of the viscometer has been considered frequently, but this has been in the nature, again, of studies with specified boundary conditions on the wall. The results of these can be found in Bird, et al, (3) and Von Wasser, et al, (37). Further in this line some very interesting work has been done by Bjorlund and Kays (5, 20) concerned with the heat transfer from rotating horizontal cylinders. Their data provides a rough way of estimating the wall temperature from which the temperature can be estimated in the gap of the viscometer. This concern with temperature is important since, as pointed out by Kimber and Linder (21), exposure of red blood cells to 50 degrees centigrade results in hemolysis. Therefore, one must be sure that the experiments are conducted under reasonable temperatures.

Another complication associated with the proper interpretation of the results is that of mixing. This problem can vary markedly with the experimental set up. The mixing of concern is not that which would be associated with turbulence necessarily, but rather that which might be associated with a secondary flow such as Taylor vorticies. As described by Taylor (35), Coles (13), and Walowit (38), the Taylor vorticies type of secondary flow will occur only in the
situation with the inner cylinder rotating. This is not too frequently encountered in more recent equipment. Walowit, though, points out that in the situation in which Taylor vorticies may occur temperature variations across the gap can enhance the possibility of Taylor vorticies formation. Mixing can occur from other sources, such as edge effects, as described by Van Wasser. These are not described in a mathematical context so that flow could be properly anticipated. But they are of the nature that one would expect flow between a buffer layer and the shear volume in the gap.

It is interesting to consider what might be found from the results of some of the capillary viscometers. Haynes\(^{(18)}\) has done a quite complete study of the blood in capillary viscometers. Charm\(^{(11)}\) has found, however, that the Poiseuille equation applies to blood when the yield stress divided by the velocity squared is less than $5 \times 10^{-4}$ and the diameter of the capillary is greater than 155 microns, and the hematocrit is greater than 40 percent. This seems to imply that the cell-free layer at the wall would have some significance. The effect of a cell-free layer has received a great amount of treatment in various fashions, by Whitmore\(^{(43)}\), Bennett\(^{(1)}\), and by Navari and Gainer\(^{(29)}\) as an attempt to estimate the effect of the sigma effect (migration of cells from the walls) on the apparent
viscosities. Navari, in particular, found very poor agreement with Poiseuille flow at high apparent viscosities. In particular, the capillary viscometer as used with very short tubes as is sometimes the case in blood work results in a steady state flow situation, but without fully developed velocity profile. Harris\(^{(17)}\) finds that in a non-steady state situation that blood is viscoelastic and definitely demonstrates an elastic term. Philippoff also finds that albumin solutions (blood has 7 percent albumin in the plasma) are viscoelastic. It again should be pointed out however, that as reported by Wells, blood is Newtonian at shearing rates of above approximately 125 inverse seconds.
EQUIPMENT

The viscometer used for the majority of the experimentation was a modified Fann Instruments Model 38A (Figure 1) in a Mooney configuration. The theory of the Mooney viscometer will be found in Appendix A. The viscometer as obtained had already had some modifications made by Nevaril\(^{27}\) to the basic instrument.

The drive mechanism of the Fann Instruments 38A is normally by shaft and gears from the variable speed electric motor to the power shaft, which drives the cup of the viscometer. This arrangement was found to be inadequate as evidenced by the brass shavings from one gear. There was also an indication from the work of Nevaril that a previous modification to increase the shear rate by changing the gearing was marginal, in that the motor was "lugging." In light of this finding, the drive was modified to a timing belt drive. This was accomplished in a direct manner by having Fann Instruments manufacture a drive shaft blank. Into this blank, after turning to radius, fifteen, 1/5-inch pitch groves were cut, providing a mating for two 3/8-inch wide, 1/5-inch pitch timing belts. To accommodate the timing belts it was necessary to enlarge the connecting shaft tunnel so that the belts could communicate to the motor without rubbing.
FIGURE 1 MODIFIED FANN VISCOMETER SHOWING CUPS AND BOBS
To power the new assembly, a Robbins and Meyers 1/2 h.p. at 5,000 rpm universal motor in a Type 4334 frame was obtained. The shaft was fitted with two 1/5-inch pitch, 3/8-inch width sheaves of 22 grooves, which had been modified by removing the hub extension and providing two lock screws through the base of the grooves to lock to the motor shaft. The motor was mounted on the vertical frame of the viscometer. The tension in the belts was controlled by shims under the motor mounts.

This motor selection with the pulley ratios used permits the cup to be turned at speeds in excess of 8,000 rpm.

The control of the speed of a universal motor can be accomplished in two convenient ways: through an auto-transformer, and through an SCR motor controller. The latter was selected as it offered the potential of greater stability, as a passive feedback is provided by which the torque of the motor is stabilized. A controller was fabricated based on the Motorola Application Note AN-198. The motor speed controller incorporated into the basic design a variable feedback resistor to increase the stability over the full range of application.

To measure the speed of revolution of the drive head, a D.C. generator type tachometer was attached to the armature shaft of the
motor through a flexible coupling. The output of the tachometer is 7 v. per 1,000 rpm. which was read on a Simpson digital voltmeter. The voltage reading plus the knowledge of the ratio of the sheaves provides the speed of the drive head.

The torque sensing mechanism of this viscometer was not modified. The torque is measured by sensing the displacement of the bob, which is connected to a linear force helical spring by a shaft passing through the hollow power head. Above the torque head, the torque shaft is attached to a protractor scale so that the angular displacement can be read directly over a range of 300 degrees. The torque shaft continues through the spring through a universal joint to the shaft of the wiper arm of a sensitive variable resistor, a Giannini Mini-Torque Potentiometer, Model 85153. This variable resistor is part of a resistance network powered by two Mallory 1.5 v. mercury cells in series, which provide a linear analog read-out of angular displacement of the torque shaft, if the input resistance is large (approximately 1 megohm) of the sensing instrument. A second potentiometer is provided for balancing the network.

The torque springs are removable and replaceable with ones of different values. This permits the operator to select a spring to provide maximum sensitivity of the torque measurement over the range to be covered in the specific experiment.
The lower portion of the power head drive shaft is provided with 1.500 x #28 threads which mate with a Lucite adaptor (Figure 2). The adaptor serves multiple functions in that it provides the mechanism for setting the bottom gap between cone and plate of the cup and bob. This setting is secured by a lock ring which is tightened by three set screws located at 120 degree spacing. The set screws provide a method in combination with the adaptor to obtain concentricity of the cup with the bob by use of the screws. The adaptor is also used to secure the cup to the drive shaft. A further advantage is obtained in the prevention of the blood from spinning up the wall of the cup.

A new aluminum cup (Figure 3) was fabricated to satisfy some apparent inadequacies in existing cups. The improvements desired were improved dimensional stability which would affect the flow in the gap if distortion were present, as is the case with plastic cups at higher angular velocities. It was also desired to improve the heat transfer characteristics of the apparatus as well as having better dimensional stability of the gap under thermal expansion.

Two different bobs were used during the course of the experiment. They both have the same construction (Figure 4). The difference in the two bobs being in the outside diameter of the cylinder
FIGURE 2  ADAPTER FROM
POWER HEADS TO CUPS

6-32 Set screws every 120°

Thread 1.500 x 28

Thread 1.52 x 24
FIGURE 3  DESIGN OF CUPS
USED ON VISCOMETERS
FIGURE 4 CONSTRUCTION DESIGN USED FOR BOB
and in the angle of the cone at the base of the cylinder (Table 1).
The bobs were constructed of 300 series stainless steel. The two
bobs with the cup provided an opportunity to investigate effects
which might be gap dependent.

<table>
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<th>Part</th>
<th>O.D. (in.)</th>
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<th>Mass g.</th>
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<td>1.387</td>
<td>126.1</td>
<td>2.480</td>
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<td>1.379</td>
<td></td>
<td>115.1</td>
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<td>1.357</td>
<td></td>
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Cup-bob combined Shearing rate Gap volume Total volume s/v Ratio

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<tr>
<th></th>
<th>Shearing rate</th>
<th>Gap volume</th>
<th>Total volume</th>
<th>s/v Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cup I + Bob I</td>
<td>18.206 sec⁻¹/RPM</td>
<td>0.4735 ml.</td>
<td>6.50 ml.</td>
<td>210.83 cm⁻¹</td>
</tr>
<tr>
<td>Cup I + Bob II</td>
<td>6.488 sec⁻¹/RPM</td>
<td>1.287 ml.</td>
<td>7.25 ml.</td>
<td>78.53 cm⁻¹</td>
</tr>
</tbody>
</table>

**Capillary Viscometer**

A simple capillary viscometer (Figure 5) was constructed
using as basic parts a Hamilton gas-type syringe No. 1010 in a
10 cc size. This syringe has a glass body with a Teflon seal on an
anodized aluminum plunger. The syringe has a Lur-Lock taper
which fits standard hypodermic needles. Interposed between the
FIGURE 5. SCHEMATIC OF CAPILLARY VISCOMETER

A — Rack for weights
B — Syringe
C — Pressure transducer
D — Hypodermic needle
E — Reservoir
syringe body and the hypodermic was a Tee Lur-Lock fitting. To the side connection of the Tee fitting a Statham Universal-type pressure gauge with a 200 diaphragm was used to sense pressure drop. This pressure was read on a Universal Transducer Read-Out Model UR-5 by Statham. The capillary tubing used was standard hypodermic needles of varying lengths and gauge sizes as needed. This permitted the plunger of the syringe to be gravity loaded. Various loadings were provided to the plunger by positioning a rack on the plunger on which lead weights could be placed.
PROCEDURE

In initiating an experiment with a concentric cylinder viscometer the first decision is what spring is to be used. The spring is then installed at the top of the torque head. The bob which is to be used is then screwed on the torque shaft. The shaft has been provided with a left-handed thread so that during the experiment the bob cannot become disengaged from the shaft. The bob is then aligned with the use of a dial indicator to the nearest five - ten thousandths of an inch. This is accomplished by gently bending the torque shaft. As the bob is rotated the cup is then slowly screwed onto the adaptor until the shoulder of the cup and adaptor are firmly positioned against each other. The adaptor is then rotated until a deflection is noted on the protractor scale. This is an indication that the cup and bob are dragging. The adaptor is then carefully rotated in the opposite direction until the protractor scale returns to zero. It is again rotated in the reverse direction until the slightest deflection is observed on the protractor scale and then backed off again the smallest amount to remove the deflection. This provides a setting for the spacing of the cone and plate. After this has been done, the cup is aligned for concentricity using a dial indicator and by alternately tightening the screws in the adaptor ring. This ring
serves two purposes in that it aligns the cup and also locks the adaptor into position. During the alignment of the cup it is filled with liquid to compensate for any buoyancy effects that might exist during the actual operation of the viscometer. During operation, the cup is filled to the level of the bottom of the adaptor approximately $6\frac{1}{2}$ ml. After filling and tightening the cup into position on the adaptor the cup is then unscrewed, rotated, and repositioned. This facilitates the removal of trapped air bubbles. Sampling is accomplished by loosening the small valve at the bottom of the cup. The sample is taken drip wise from this valve after discarding the first draft.

As the cup and bob are very carefully machined it is possible to directly calculate the value of shearing rate as a function of rpm from the dimensions. It is necessary to calibrate the torque springs. The torque springs are calibrated by using known standards fluids. This is done by comparing the known shearing rate and known viscosity against the torque readout of the viscometer (Figure 6). Specifically, the known fluids used are Dow Corning Type 200 Silicone Oils. (See Table 2 for the values of the springs with the confidence limits derived from the calibration tests.)
F10 Spring
Dow Corning Fluid 200

FIGURE 6 CALIBRATION CURVE FOR F10 SPRING
TABLE II

<table>
<thead>
<tr>
<th>Spring</th>
<th>Determination</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$F_4$</td>
<td>6</td>
<td>$20.83 \pm 0.61$ dynes/cm$^2$/0</td>
</tr>
<tr>
<td>$F_{10}$</td>
<td>6</td>
<td>$48.80 \pm 0.82$ dynes/cm$^2$/0</td>
</tr>
</tbody>
</table>

In this work it is evident that to know the shearing rate in the concentric cylinder viscometer one must have an accurate measure of the rotational rate of the power drive unit. As was indicated in the section on equipment, an electrical tachometer was provided to read the rate of revolution of the electric drive motor. And from this the rpm of the drive shaft was calculated. To confirm that the electrical tachometer was accurate and linear over the range of use, the readout from this device was compared with the rate of revolution indicated by a strobotach made by Jacquet Tachometer. The agreement of all three was well within the accuracy of the devices being used. And the tachometer was found to be linear over the range of 0-8,000 rpm.

As this work deals mainly with the use of the concentric cylinder viscometer with blood it was felt that the blood used in these experiments should be used in a condition that was as reproducible as possible. Therefore, in all reported experiments the
blood as used was collected in ACD, NIH formula A, by venapuncture into a plastic collection vessel. This may have been syringe or plastic bag. All blood used in these tests was from fasting, healthy male donors. The blood was used within 24 hours from time of collection, and all tests on the effects of shearing rate on the blood in which hemolysis measurements were conducted were made at ambient temperature, which in this laboratory is approximately 21°C plus or minus one degree. The blood was allowed to slowly come to ambient before use and care was taken that the temperature of the blood was maintained at a constant level. Further care was taken to see that in no way was the blood subjected to thermal or mechanical shocks outside the viscometer.

Before an experiment was run with blood in the viscometer, all surfaces of the viscometer which would come in contact with blood were siliconized with Clay Adams Siliclad. The procedure followed for silicoating the surfaces was as recommended by the manufacturer of Siliclad. Further in the procedure for use with blood in the viscometer great care was taken to remove any bubbles that might reside between the cup and bob. This was done, as previously stated, by loosening the cup and bob and tightening again followed by a withdrawal of a couple of drops from the valve.

The sampling
procedure followed with blood was to open the stop valve at the bottom of the cup after the termination of the run. The sample was then taken drop wise, after discarding the first drop. Calibration indicate that the size of the drop was approximately one-twentieth of a ml. This information provided the technique for sizing the sample. From the blood sample taken, a small drop would be used for making a blood smear. The blood smear, after being allowed to dry, would be fixed in absolute methyl alcohol. Following this, the slide would be stained with Wright stain. The remaining blood sample which was taken in a conical plastic centrifuge tube would be centrifuged under 2,000 G for ten seconds in a Sorval GLC-1 centrifuge. The plasma would then be separated from the red cells and would be subjected to a plasma hemoglobin test based upon the benzidine method (see Appendix C). Also, on the control blood that was used for the test hematocrit determinations and total hemoglobin would be measured to provide a basis for assaying the level of damage.

In the experiments designed to measure the level of mixing of the gap sample with the buffer blood in the space above the high shear area in the viscometer, the blood was labeled in two fashions. The plasma was labeled with \textsuperscript{125}I Serum Albumin Risa. This provided a measure of the level of mixing of the plasma components of the blood. The red cells were labeled with \textsuperscript{51}Cr using the normal
chromium tagging procedure. This provided a measure of the mixing of the red cells between the two portions in the viscometer. The samples were taken drop wise with one drop to a sample tube for the first twenty drops, approximately twice the volume of the gap. After this, the samples were taken ten drops to a tube. The samples so collected were then counted in a well-type scintillation counter. Two different window and gate settings were used to separately count the different labels, in this way allowing a separation of the mixing effects. In performing the labeling experiments a different technique had to be used for introducing the blood samples into the viscometer. The technique that was used was to add 5 ml. of unlabeled blood to the cup of the viscometer. The procedure for removing the bubbles was then followed. Through a tap hole in the adaptor ½ cc of labeled blood was then introduced. One cc of unlabeled blood was then added rapidly to provide some mixing through the hole in the adaptor. This technique provided a way of introducing a radioactive label to the buffer portion of the blood without introducing any label to the sample of the blood in the gap of the viscometer. Following this, a normal sequence for a run for a hemolysis study would be made.

In the heat generation portion of the experimentation a silicone oil, Dow Corning Type 200, in a 5 centistroke value was used rather than blood. This oil was selected because over the temperature range
of interest it has well established characteristics which are linear in the log portion of the curve. The characteristics of this oil were confirmed by thermostating the viscometer at various temperatures and then measuring the viscosity of the oil as a function of temperature (see Figure 7). The procedure used for measuring the temperature rise in the gap of the viscometer was based upon the torque decay as measured by the viscometer as heat is generated in the gap. Of course, this provides only an average temperature based on the average viscosity in the gap. For these experiments a Honeywell Electronic 19 Recorder was connected to the torque readout bridge. In this way it was possible to obtain a direct recording as a function of time of the torque. For this to be meaningful, the motor speed was maintained at a constant level during the experiments. As indicated in the equipment portion, the motor speed was sensed by an electric tachometer but manually adjusted. Procedure further used in this experimentation was to load a six and one half ml. sample of silicone oil into the viscometer. The bubble removing procedure was then followed. The viscometer was then started at the rpm that was desired. The torque decay was then followed using the recorder until the steady state was reached as determined by no change in torque in three minutes. Care was taken during these experiments to be sure that the oil in the viscometer as well as the cup and bob
Figure 7: Viscosity of silicone standard as a function of temperature.

Dow Corning Fluid 200
had returned to ambient temperature before a new experiment was started. An indication of this could be obtained by measuring the viscosity of the oil at very low shearing rate. No cross flow was provided to assist in the cooling of the cup during the heat generation experiments.

For the use of the capillary viscometer for shear stress studies of blood it was necessary to calibrate in a very careful way the diameter of the needles. This was done for comparison purposes by several techniques. The first of these consists of direct measurement of the inside diameter of the needles by the use of a microscope. This was done specifically by measuring the largest I.D. of the bevel of the needle using graduated objectives on the microscope. The second technique used to give a value of the inside diameter of the needle was through the use of calibration fluids under a known pressure head with the measurement of the flow rate from the tube. A third method used for this was to weigh a known length of hypodermic needle tubing and from this, with the specific gravity of the steel used in the manufacture of the needles, it is possible to calculate the void volume which in turn gives the inside diameter. The results of the calibration of the needles used with the values provided by the manufacturer are presented in Table 3.
TABLE III

Capillary Needles

<table>
<thead>
<tr>
<th>Needle</th>
<th>Manufacture</th>
<th>Optical</th>
<th>Mass</th>
<th>Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>23 G</td>
<td>0.33 mm</td>
<td>0.332 ± .001</td>
<td>0.334 ± .003</td>
<td>0.34 ± .02</td>
</tr>
<tr>
<td>25 G</td>
<td>0.25 mm</td>
<td>0.251 ± .001</td>
<td>0.2495 ± .002</td>
<td>0.27 ± .04</td>
</tr>
</tbody>
</table>

The pressure transducer which was used for measuring the pressure between the syringe and the needle providing a measure of the pressure at the entrance to the capillary was calibrated statically through the use of a mercury manometer. The readings provided by the pressure transducer were found to agree within the readability of the dial with the pressures provided for calibration from the mercury manometer.

The capillary viscometer was used in two configurations. First, in the configuration with the syringe and needles mounted in a vertical position and pressures provided by weights placed on the plunger of the syringe. When used in this configuration, calibration was obtained from the use of standard fluids and out-flow viscometry. From this it was possible to determine the wall shear stress when blood was used in the viscometer. The second configuration that was used was that in which the pressure transducer was connected between the needle and syringe. In this method it was possible to
have a more direct measure of the pressure drop through the syringe. In this type of procedure it is necessary to correct for end effects. This correction for end effects was made using the Hagenbach technique (Appendix B).

In the use of blood in the capillary viscometer the specific setup or procedure used was one in which slightly more than 5 ml. of blood was slowly drawn into the syringe with the needle removed. The syringe and needle with or without pressure transducer were then rejoined. The syringe was pointed upward and all bubbles were carefully expelled, leaving 5 ml. The syringe and needle were then returned to a position in which they pointed vertically downward. The viscometer was then so positioned that the needle projected through the surface of 2 ml. of blood placed in a polypropylene weighing bottle of a capacity of 15 ml. This was done so that there would not be a jetting through an air interface. At this point the desired weights were placed on the plunger of the syringe and the time for outflow was measured. In the case where the pressure transducer was used, the pressure was then recorded.

In the experiments in which albumin was added to the blood, the procedure that was followed was first to centrifuge the blood as collected in ACD in 50 ml. polycarbonate centrifuge tubes for thirty minutes at a G-force of 2600. After this had been done the plasma
was very carefully separated from the cells and placed in an Erlenmeyer flask. This flask had previously been silicoated. To the plasma a quantity of human albumin was added obtained from Calbiochem Grade B to provide an added concentration of albumin of 34 grams per hundred ml. in the plasma. To dissolve the albumin in the plasma it was necessary to use a magnetic stirring bar, Teflon coated, and a stirring plate. The sample was then stirred covered until all solid albumin was dissolved in the plasma. After the albumin was dissolved the flask was allowed to rest so that the bubbles could disperse. At this point the separated red cells were added and carefully mixed. The sample could now, if desired, be centrifuged to obtain a fraction of neutrally buoyant cells. Because of this, during any sampling procedure in which a sample of plasma was desired, it was necessary to add one ml. of isotonic sodium chloride for every five ml. of albuminized blood sample to be separated by centrifugation. To prepare a slide of albuminized blood in a dry preparation it was necessary to first separate the cells and then re-suspend the cells in isotonic saline before a slide could be made. If not, the sample would flake from the slide.

In deciding to use albumin in this high concentration it was
necessary to be sure that all albumin had dissolved. To check that this was the situation, a wet preparation was observed under a microscope at the magnification of 500 diameters. The results of this observation was the finding that no crystals of albumin were visible in a bright field or in a phase field. Further, upon observation of the red cells it was found that there were no larger aggregates apparent than groups of three. And this is not uncommon situation in the un-albuminized blood as handled in this laboratory.
RESULTS

1. Surface to Volume Studies

To resolve the recurring question as to the mode of destruction of red cells in a flow field, that is, whether the mechanism is of a surface interaction or a bulk process, the destruction of red cells (human) as measured by hemolysis was observed under the parameter of variation of surface to volume ratio while under shear. This was done following the previously described procedure for shearing blood in the Mooney type viscometer by observing the relationship of the shear stress versus cell damage curves when the bobs were changed. The interchange of the bobs provides more than a change in gap which results in a change in shearing rate per rpm. It provides also a change in the surface to volume ratio of the individual combination of cup and bob. This is reflected in Table 4:

<table>
<thead>
<tr>
<th>Cup and Bob Combination</th>
<th>Gap Radius of Bob (cm)</th>
<th>Shear Area cm²</th>
<th>Shear Volume cm³</th>
<th>Area/Volume Ratio cm⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aluminum cup &amp; large bob</td>
<td>.010 cm 1.751 cm</td>
<td>99.830</td>
<td>.4735</td>
<td>210.834</td>
</tr>
<tr>
<td>Aluminum cup &amp; small bob</td>
<td>.038 cm 1.723</td>
<td>101.069</td>
<td>1.2870</td>
<td>78.531</td>
</tr>
</tbody>
</table>

Ratio total = 2.68
In Figure 8 curves, each representing a different donor, of hemolysis versus shear rate for three different runs in which the bobs were changed during the runs are presented; a direct comparison can be made of the damage under the two different shear situations. As can be observed, there is little difference in the percent of red cell damage in the large and small gap configurations in the experiment, even though there is a surface to volume ratio difference of 2.58 to 1. In Figure 9, the results from these experiments are replotted with a change of axis. In this case the plot is of hemolysis per square centimeter versus shear stress. In this it can be observed that the result of the new axis is to separate the results of the individual experiments in such a way that it is evident that the damage per square centimeter is smaller in the larger gap. It is quite evident there is good agreement between the two different shearing situations, strongly suggesting that the major force of action is one of a volume process rather than a surface process. This does not deny that cells can be damaged at a surface, but rather states that under the circumstances investigated in these experiments, i.e. that of high shear stress, the major factor of importance is that of shear stress rather than surface area exposed to the blood. To give further substantiation to this
Figure 8: Percent of RBC Hemolysed vs Shear Stress (Dynes/cm²)

Surface/Volume Ratio
- Δ 210.8 cm⁻¹
- ○ 78.5 cm⁻¹
PER UNIT AREA AS A FUNCTION OF SHEAR STRESS

FIGURE 9  HEMOGLOBIN RELEASED
PER UNIT AREA AS A FUNCTION OF SHEAR STRESS
thesis, the previous runs were also done under the situation in which the cells were partially neutrally buoyant; that is, in a heavily albuminized plasma substrate as described in the procedures. The results of these experiments as indicated in the two adjoining Figures 10 & 11 strongly confirm the results with normal blood. The albuminized blood releaves the possibility that the requirement for surface interaction is related to the velocity of the particles under shear. Because in the albuminized situation the damage level is higher and the relative velocities are lower by the ratio of the viscosities which is of the order of three. This gives further support to the fact that the major process is one of shear in the bulk phase rather than an interaction at the surface.
FIGURE 10  % RBC's HEMOLYSISED AS A FUNCTION OF SHEAR STRESS
Figure II: Hemoglobin released per unit area as a function of shear stress.

- O 78.5 m\(^{-1}\)
- Δ 210.8 cm\(^{-1}\)

34% Albumin in plasma
2. **Temperature Studies**

As blood is sensitive to thermal destruction it is necessary to control or at least to predict the temperature rise of a blood sample during shear for the planning of meaningful experiments. In the available viscometer a direct temperature measure is not possible. The inner wall or bob is effectively adiabatic at steady state. In this situation the temperature gradient relative to the inner wall of the cup is

\[
Ty - To = \mu \frac{V^2}{2K} (1 - \frac{V^2}{d^2})
\]  

or the temperature difference across the gap is

\[
\Delta T = \mu \frac{V^2}{2K}
\]  

For the most severe case considered experimentally (\(10^5\) sec\(^{-1}\)) based on the coefficient of thermal conductivity of Dow Corning Type 200-5CS silicone oil, the \(\Delta T\) across the 0.1 mm gap is less than 2.5°C which corresponds to a viscosity change of approximately 0.15 cp across the gap. This implies that the change in the velocity distribution will be small. For a similar consideration on blood the \(\Delta T\) would be less than 0.5°C.

The preceding discussion suggests that since viscosity is a function of temperature (See Figure 7) the measurement of torque may be used to determine temperature. This provides information
on the steady state temperature rise in the gap as well as the transient behavior (Figure 12).

From the curves obtained at different shearing rates a plot of temperature rise at steady state above ambient versus shearing rate is presented in Figure 13. A different representation of this same data might be as temperature rise versus heat generation.

The blood metal film coefficient can be estimated by the Ditlus-Boelter equation or by the Chilton, Drew and Jebens equation. Either of these techniques gives the result of a $\Delta T$ across the film of less than $0.02^\circ C$ for the largest generation rate considered. This really suggests that $h_j$ (the blood metal film coefficient) can be neglected. Using the conductivity of the cup material, the air-metal film (see Figure 14) coefficient can be calculated thusly:

$$S = UA\Delta T = U_0 (2\pi R_0 L)\Delta T$$

$$\frac{1}{UA} = \frac{1}{h_i A_i} + Rw + \frac{1}{h_o A_o}$$

(3)

This can then be presented as a plot of heat transfer coefficient versus velocity (Figure 15). A more useful representation might be Nusselt number versus Reynolds number based on the cup diameter (Figure 16). Kays and Bjourland have considered the case of heat transfer from a rotating horizontal cylinder. Their data is similarly as the dotted curve but this is a marked different geometry.
Ambient 21.5°C
Shear rate $2.07 \times 10^5$ sec$^{-1}$

![Graph showing the decay of torque over time]

$\tau \times 10^{-3}$ Dynes / cm$^2$

Time (min)

\[\text{FIGURE 12} \quad \text{TYPICAL TORQUE DECAY CURVE WITH SILICONE OIL}\]
FIGURE 13  TEMPERATURE RISE AS A FUNCTION OF SHEARING CONDITIONS FOR CUP AND BOB CONDITION I
FIGURE 14  SCHEMATIC FOR STEADY STATE HEAT TRANSFER IN CONCENTRIC CYLINDER VISCOMETER
FIGURE 15 HEAT TRANSFER FILM COEFFICIENT VERSUS SHEAR RATE
FIGURE 16  NUSSETT NUMBER VERSUS REYNOLDS NUMBER FOR A VERTICAL CYLINDER
Since it is desirable to experiment outside the apparent safe range of shearing rate for an acceptable steady state temperature rise, the area of transient rise is of interest. As an example, Figure 17 gives the temperature rise after two minutes. It can be seen that the temperature rise is markedly below the asymptotic level. A simple model is predict the temperature in the gap follows. (Figure 18).

As the ratios of the various radii involved approach unity, the heat transfer problem can be reduced to that of conduction in one direction. The internal surface of the bob is assumed to be adiabatic as it does not communicate with the surroundings. In the equations presented the Bs are ratios of (Areas) (film coefficients) divided by (masses)(heat capacities). The $\mathcal{C}$ is a heat generation term divided by the appropriate (mass)(heat capacity).

$$\frac{dT_1}{dt} = B_1 (T_2 - T_1)$$

$$\frac{dT_2}{dt} = B_2 (T_1 - T_2) + B_3 (T_3 - T_2) + \mathcal{C} \beta T_2$$

$$\frac{dT_3}{dt} = B_3 (T_2 - T_3) + B_5 (T_0 - T_3)$$

The heat generation term can be expanded in a Taylor series and then, taking the first two terms, this should be good over a small range of temperature change, giving a linear system of equations. These equations can now be transformed using Laplace transforms and
2 minute experiment
--- model

FIGURE 17
TEMPERATURE RISE AT 2 MINUTES VERSUS SHEARING RATE

ΔT (°C)  20  10  0

\( \dot{\gamma} \times 10^{-4} \text{ (sec}^{-1}) \)

0  5  10  15  20
FIGURE 18. SCHEMATIC FOR NON-STeady STATE HEAT GENERATION IN GAP
the boundary conditions
\[ t = 0 : T_1 = T_2 = T_3 = T_0 \]  \hspace{1cm} (5)
to give
\[
\begin{align*}
S T_1 &= B_1 T_2 - B_1 T_1 + T_0 \\
S T_2 &= B_2 T_1 - B_2 T_1 + B_3 T_3 - B_3 T_2 + T_0 + \frac{\alpha}{S} + B T_2 \\
S T_3 &= B_4 T_2 - B_4 T_3 + \left( \frac{B_5}{S} + 1 \right) T_0 - B_5 T_3
\end{align*}
\]  \hspace{1cm} (6)

Solving for the various temperatures and inverting we get the temperature as a function of time.
\[ T_i = f_i (B_3) e^{d_1 t} + g_i (B_3) e^{b t} + j_i (B_3) e^{c t} + \lambda_i (B_3) e^{B T} \]  \hspace{1cm} (7)

The preceding equations are somewhat cumbersome for easy use. If the conditions of a large liquid-solid film coefficient and small resistance in the cup as compared to the air-solid interface a lumped parameter model can be used with excellent agreement as follows
\[
\left[ m_1 C_{p_1} + m_2 C_{p_2} + m_3 C_{p_3} \right] \frac{dT_2}{dt} = h_1 A_1 \left( T_0 - T_2 \right) + \alpha e^{B T_2} \]  \hspace{1cm} (8)

In using the derivations the coefficient for the density and specific heat term in the gap have to be slightly modified to represent an effective term if mixing is present. Using the mixing experiments as reported for this viscometric geometry and empirical effectiveness factor which is a function of shearing rate is used. This provides nothing more than an effective heat dissipation term.
Ambient 21.5°C
Shear stress $2.07 \times 10^5$ sec$^{-1}$
Dow Fluid 200

--- Experimental
--- Model

**FIGURE 19** $\Delta T$ AS A FUNCTION OF TIME
Figure 19 provides typical calculated curves of the temperature rise of the gap, and as a function of time. The dashed line is the experimental curve for the gap temperature. The plot is made in such a way as to be directly comparable to Figure 12.

This development is provided as a working design criteria rather than a precise analysis and solution.
3. **Mixing Studies**

For studies to be meaningful on the extent of damage to RBC's, one must be sure of the true volume of blood under study. This may not be a simple problem if a buffer layer is used to separate the blood under shear from an air interface, because interchange can take place between the two volumes.

To make an initial consideration it was decided to compare the plasma hemoglobin in the buffer layer to the plasma hemoglobin in the gap. The measured concentrations are presented in Figure 20. It will be observed that even in the highest shear stress studied the concentration of plasma hemoglobin in the buffer layer is only about 25% of concentration in the gap. This does not appear to be a very serious problem until it is recalled that the volume of the buffer layer is approximately twelve times the volume of the gap. This means that the mass of plasma hemoglobin in the buffer layer is three times the mass in the gap at a shear stress of 4000 dynes/cm$^2$. A comparison of the mass of plasma hemoglobin is made in Figure 21. It will be observed that the mass of plasma hemoglobin in the buffer layer is certainly significant as compared to the levels in the gap. This would require significant correction to the directly measured plasma hemoglobin in the buffer layer if derived by mixing from the gap alone.
FIGURE 20 COMPARISON OF PLASMA HEMOGLOBIN LEVELS IN GAP AND BUFFER LAYER
Figure 21: Comparison of mass of plasma hemoglobin in gap and buffer layer.
FIGURE 22, COMPARISON OF RADIOACTIVITY
\( ^{+51} \text{Cr} \) MIXED INTO GAP FROM BUFFER
LAYER AS A FUNCTION OF GAP POSITION
FIGURE 23 COMPARISON OF $^{125}$I MIXED INTO GAP AS FUNCTION OF GAP POSITION
To resolve the true mixing, a radioisotope tagging system (I$^{125}$ to tag the plasma and Cr$^{51}$ to tag the red cells) was used to analyze the situation. The results, normalized by the average count per incremental volume, which would be the value for total mixing, are presented for the red cells in Figure 22 and for the plasma Figure 23.

The abscissa in these figures actually is drop number. Each drop contains about 0.05 ml. The gap was drained one drop at a time and the radiation from each drop was counted. The ordinate is the count per unit volume so measured divided by the overall average count per unit volume. Hence, the count would be constant at unity if we had perfect mixing. The count would be uniform at zero if there were no mixing and sampling by piston flow since the tag is applied to the uppermost part of the buffer layer. If the flow from the gap during sampling follows the no slip at the walls criteria and if Newtonian fluid from the buffer layer would appear before the gap is drained. The anticipated behavior of this flow is indicated as the zero mixing curve in these graphs. If this were the true situation, one would expect an increase of slope associated with the measured curves at breakthrough. This is not seen and suggests incomplete mixing of the buffer layer near the gap. The effect of this would be to compensate for the breakthrough.
FIGURE 24 MIXING RATIO AS A FUNCTION OF SHEAR STRESS
It is also evident that the level of mixing is dependent on the shearing stress. If the average extent of mixing is considered for the total volume in the gap, the dependence appears in Figure 24 where the ordinate is average counts per unit volume in the gap divided by the average counts per unit volumes for the whole system. It must, of course, be remembered that this is for the specific geometry of the viscometer described earlier.

Mixing would have an influence in two fashions: the first is to mask in the apparent hemolysis rate in the gap, and the second is to enhance the dissipation of heat generated in the gap by viscous heating.

To evaluate the possibility of hemolysis in the buffer layer, a simple model, that of mixing between two tanks connected by two pipes, can be considered. With the assumption of constant volumes, the equations which describe this are

\[
\frac{dc_1}{dt} = \frac{q}{V_1} (C_2 - C_1) + \frac{k_1}{V_1} \tag{9}
\]

for the gap, and

\[
\frac{dc_1}{dt} = \frac{q}{V_2} (C_1 - C_2) + \frac{k_2}{V_2} \tag{10}
\]

for the buffer layer.

If \(k_1\) and \(k_2\) are set equal to zero which would represent no generation, the value of the mixing flow, \(q\), can be evaluated using the radioisotope data. The values of \(q\) as a function of shearing stress can be seen in the Table 5.
TABLE 5

<table>
<thead>
<tr>
<th>τ</th>
<th>q in ml/min. from the Cr\textsuperscript{51} tagging</th>
<th>q in ml/min. from \textsuperscript{1\textsubscript{25}}I tagging</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>0.2 \times 10^{-5}</td>
<td>0.2 \times 10^{-5}</td>
</tr>
<tr>
<td>1000</td>
<td>0.120</td>
<td>0.106</td>
</tr>
<tr>
<td>2000</td>
<td>0.133</td>
<td>0.120</td>
</tr>
<tr>
<td>3000</td>
<td>0.131</td>
<td>---</td>
</tr>
<tr>
<td>4000</td>
<td>0.196</td>
<td>0.180</td>
</tr>
</tbody>
</table>

The values of q presented here indicate that the mixing flows are not significantly different for the Cr\textsuperscript{51} tagged cells and for the \textsuperscript{1\textsubscript{25}}I tagged plasma. Further, the maximum mixing flow could not account for enough mixing to provide the plasma hemoglobin levels in the buffer layers as less than one gap volume turnover would result during the normal two minute experiments. Hence, there must be hemolysis in the buffer layer.

Using the calculated values of q and the initial conditions of zero concentration of free hemoglobin which is a good approximation the hemolysis rates can be calculated using the data in Figure 20. (See Table 6).
This provides the very interesting result that the generation of plasma is greater in some cases in the buffer layer than in the gap. If the generation rates are considered on a unit volume basis, the rate of generation per unit volume in the gap is always higher than in the buffer over the range of shear stresses studied.

To evaluate the effects of these results on the actual hemoglobin measurements, a comparison can be made using the concentration predicted by the model and the amount generated as indicated by the rate constants.

That is, compare

$$ C = \frac{v_1 v_2^2 - v_1 v_2 k_2}{q (v_1 + v_2)^2} \left( 1 - e^{-\frac{q}{v_1 v_2}} \left( \frac{v_1 + v_2}{v_1 v_2} \right)^t \right) + \frac{k_1 + k_2}{v_2 + v_2} \ t \quad (11) $$

and

$$ C_1 = \frac{1}{v_1} \int_0^t k_1 \ dt \quad (12) $$
for the case of $t = 2$ minutes the results are presented in Table 7.

**TABLE 7**

<table>
<thead>
<tr>
<th>$\tau$</th>
<th>Fraction Hb remaining in Gap</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>1.000</td>
</tr>
<tr>
<td>1000</td>
<td>.98</td>
</tr>
<tr>
<td>2000</td>
<td>.91</td>
</tr>
<tr>
<td>3000</td>
<td>.88</td>
</tr>
<tr>
<td>4000</td>
<td>.797</td>
</tr>
</tbody>
</table>

The previous table provides the information necessary to correct the measured plasma hemoglobin concentrations for the gap. It will be noted that the correction is pleasantly not very large for this geometry. If $f$ represents the fraction of plasma hemoglobin remaining in the gap, then the true concentration $c_f$ can be calculated simply by

$$c_f = \frac{c_l}{f}$$

(13)

The fortunate case of having small correction factors for the geometry considered is the result of a balancing of hemolysis in the buffer, mixing rates and hemolysis rate in the gap. The hemolysis in the buffer layer is of an unexplained nature but could well result from foaming or frothing. It should be noted that in the areas of the
critical shear stress the generation rate in the buffer layer is very small and would not mask the critical shear stress.
4. **Centrifugal Studies**

As a rotating system is used in the investigation of the shear effects on blood the possibility of an effect from the centrifugal field must be considered. To separate the shear effects from any effect that might result from centrifuging the cells to the cup wall the hemolysis rate of normal blood was compared to heavily albuminized blood in which the specific gravity of the cells is approximately that of plasma.

In the small gap the results of this comparison (Figure 25) are that, even though the centrifugal effect on the cells has been markedly reduced (through higher viscosity and the relative density approaching neutral buoyancy) the hemolysis rate was of the same order as normal blood and even elevated.

The addition of the albumin results in part of the cells being neutrally buoyant, part heavier and part less dense than the plasma. Donor RK had a distribution of 10-50-30 in the order listed above. GP had a distribution of 10-80-10 and DS had 10-50-40.

This experiment was run also with the large gap configuration with very similar results (Figure 26). The use of the large gap configuration with the corresponding higher rotational rates for the same shear stress strongly indicates that the centrifugal field does not effect
Figure 25: Comparison of RBC damage as a function of shear stress in 0.1/mm gap.
FIGURE 26 COMPARISON OF RBC DAMAGE AS A FUNCTION OF SHEAR STRESS IN .38mm GAP

- Albumin 34%
- Normal
FIGURE 27

RBC DAMAGE IN CAPILLARY AS A FUNCTION OF WALL SHEAR STRESS

% RBCs HEMOLYSED

τ \times 10^{-3} (Dunes/cm^2)

μ = 3.7 cp

μ = 190 - 259 cp

Normal Blood

Albumin Added 34% ∆
FIGURE 28 VARIATION OF VISCOSITY WITH SHEARING RATE

- O Normal Blood
- △ Albumin 34% Blood
- □ Normal Plasma
- ○ Albumin 34% Plasma

\[ \mu \text{ (cp)} \]

\[ \gamma \text{ (sec}^{-1}) \]

50 40 30 20 10 0

1000 100 10 1
the critical shear stress for hemolysis. Further the hemolysis rates are not effected significantly by the centrifugal field.

To resolve the possibility of albumin affecting the shear behavior of red blood cells, some simple capillary experiments were conducted. The results of these (Figure 27) are somewhat disturbing in that now the effect of shear on the two samples of blood has been reversed. The presentation, however, has been made on the assumption of inelastic behavior, which may well be inappropriate. As a suggestion of this, the apparent viscosity of the albuminized blood is approximately three times higher than in the Mooney type viscometer.

To check the viscometric behavior of the two types of systems used for a more careful measure, they were run on a Wissenberg Rheogoniometer with the plasma samples (Figure 28). The viscosities are in agreement with the Mooney configuration measurements. The Newtonian behavior above 100 sec$^{-1}$ should also be noted.

An explanation of the capillary effect is possible and that is that the damage in this system is done in the jet. This is appealing in that the maximum shear stress is proportional to the velocity squared in a jet. Further, Blackshear has reported no damage unless the velocity is greater than 1000 cm/sec. which is obtained by the normal blood but not with albumin added. It should be noted
further that the entrance length \((L_e = 0.0350 \text{ Re})^{(3)}\) is significant
approaching one-third of \(L\) for the higher shear stress.

The important point of the capillary measurements is that
over the shear stress range studied in the concentric viscometer no
difference is seen in the behavior of the blood with or without Albumin.
5. **Morphology**

The results relative to the effect of surface to volume ratio and centrifugal forces on red cell-shear interaction have been based solely on hemolysis data. To further expand the basis for evaluating the effects of interest, the change in cell morphology was observed by determining the percentage of altered cells on the basis of one thousand cells per data point.

The results of this type of analysis is shown in Figure 29 for the situation of varying surface to volume ratio and also for the consideration of change in albumin concentration. Qualitatively, if compared to Figures 8-11 it can be seen that in the effect of change in surface to volume ratio is of little importance in the high shear region. This certainly provides additional bases for considering the bulk process of shear stress of prime concern in red cell damage at high shear stresses.

It should further be noted that the concept of a critical shear still holds though shifted to the left. This shift is certainly not unexpected as one would feel that it is easier to damage a cell than to grossly rupture.

Again, as in the hemolysis data, the damage rate with albumin present is higher than without confirming the results presented for
Figure 29: Morphology changed as a function of shear stress.

- △ Normal Blood Small Gap
- ○ Large Gap Normal Blood
- □ Albumin Added Sb
- ○ Albumin Added LG

% Modified RBC's vs. τ x 10^{-3}
hemolysis in the section, centrifugal studies.

It should be stressed that this type of analysis is subject to errors resulting from variation in slide preparation and is by its nature somewhat subjective.
CONCLUSIONS

High shear stresses above the critical shear stress (approximately 1500 synes/cm$^2$) are of far greater significance in red cell damage than surface effects. This was confirmed for hemolysis and for cell alteration. This does not deny the influence of surfaces but rather indicates it to be of second order for high shear stresses.

Mixing does occur between the blood in the gap of a concentric cylinder viscometer and the buffer layer above. From a simple model it is possible to calculate the extent of mixing using radioactivity tagging measurements. It is further possible to calculate the hemolysis rates in the gap and buffer layer. These calculations show that hemolysis also takes place in the buffer layer at very significant rates. From this data it has been shown that previously reported results subscribe hemolysis levels that are too low. Mixing does not effect the critical shear stress.

Film coefficients for the rotating outer cylinder configuration were measured. From this with a simple model the temperature rise in the shear gap can be predicted so that experiments can be designed with confidence.

The effect of the centrifugal field on hemolysis was studied and it was found not to effect the critical shearing stress and only
slightly to affect the hemolysis rate in a concentric cylinder viscometer.

Hemolysis at high shear stresses were found to be independent of shearing rate in the capillary viscometer to levels of 6000 dynes per cm².
BIBLIOGRAPHY


APPENDIX A

THEORETICAL BASIS OF THE MOONEY VISCOMETER

The Mooney Viscometer is a concentric cylinder viscometer with the bottom of the inner cylinder cut to the configuration of a cone so that when the inner cylinder is properly spaced in relation to the bottom of the outer cylinder, and the angle of the cone properly selected, the shear stresses will be the same between the cylinders and at the bottom. The addition of the cone to the bottom of the concentric cylinder configuration serves to eliminate the end effect at the bottom.

The derivation of the equations governing this arrangement will be considered in two parts with the matching discussed afterwards.

First, consider the concentric cylinder and then the cone and plate portions of the arrangement.

This derivation will assume that blood is Newtonian, which is a good assumption at shearing rates higher than 100 sec\(^{-1}\). And as this level is far below those used in the experiments, this is certainly justified. Assume that the outer cylinder rotates with angular velocity (Figure A1) and the inner cylinder is stationary. This configuration suggests cylindrical coordinates. The velocities
FIGURE A1. CONFIGURATION FOR CONCENTRIC CYLINDER VISCOMETER WITH FIXED INNER CYLINDER
in the \( r \) and \( z \) direction are zero as is the pressure gradient in the \( \Theta \) direction. With this the equations of motion become

\[
- \rho \frac{V^2}{V} = - \frac{\partial P}{\partial r} \quad (A1)
\]

\[
\frac{d}{dr} \left[ \frac{1}{r} \frac{d}{dr} (r V \Theta) \right] = 0 \quad (A2)
\]

\[
\rho gz = \frac{dP}{dz} = 0 \quad (A3)
\]

With the assumption of no slip at the wall, Equation A2 can be integrated twice with the boundary conditions

\[
V \Theta = 0 \text{ at } r = KR \quad (A4)
\]

\[
V \Theta = \Omega o R \text{ at } r = R
\]

to yield

\[
V \Theta = \frac{1}{r} \left( \frac{K^2}{K^2 - 1} \right) \Omega o R^2 - \left( \frac{1}{1 - K^2} \right) \Omega o R \quad (A5)
\]

which is the velocity distribution across the gap.

Now the shear stress for this situation is

\[
\tau r \Theta = -\mu \left[ r \frac{\partial}{\partial r} \left( \frac{V \Theta}{V} \right) \right] \quad (A6)
\]

Substituting \( V \Theta \) from Equation A5 into A6 and differentiating yields

\[
\tau r \Theta = -\mu o R^2 \left( \frac{1}{r^2} \right) \left( \frac{K^2}{K^2 - 1} \right) \quad (A7)
\]

As can be seen from this, the dependence of \( \tau \) on \( r \) is very small as \( K \) approaches unity. That is, the shear stress across the gap approaches a constant as \( K \) approaches 1.

For the cone and plate portion of the cup and bob, spherical geometry will be used (Figure A2). Blood will again be considered
to be Newtonian. The plate will rotate with angular velocity \( \Omega_0 \) and the cone is stationary. The angle between the platens is \( \Theta_C \). It is further assumed that the flow is entirely tangential, giving

\[
V_r = V \Theta = 0
\]

and

\[

V \phi = r \Omega (\Theta) \sin \Theta
\]

The components of the equation of motion become

\[
- \rho r \Omega^2 (\Theta) \sin^2 \Theta = - \frac{\partial P}{\partial r} \tag{A8}
\]

\[
- \rho r \Omega^2 (\Theta) \sin \Theta \cos \Theta = \frac{1}{2} \frac{\partial P}{\partial \Theta} \tag{A9}
\]

and

\[
\frac{\partial \tau}{\partial \Theta} \phi = -2 \tau \Theta \phi \cot \Theta \tag{A10}
\]

Equation A11 may be integrated to give

\[
\tau \Theta \phi = \frac{C}{\sin^2 \Theta} \tag{A12}
\]

where \( C \) is a constant.

Taking the relative difference in shear stress at the plate

\[
(\Theta = \pi/2) \quad \text{and at the cup } (\Theta = (\pi/2) + \Theta_C)
\]

\[
\tau \Theta \phi [\pi/2 + \Theta_C] - \Theta \phi (\pi/2) = \tan^2 \Theta C \tag{A13}
\]

From Equation A13 it is apparent that if \( \Theta C \) is small then an assumption of uniform shear stress and hence shear rate throughout the fluid is very good.

Equation A11 can now be written

\[
\frac{\partial \tau \Theta \phi}{\partial \Theta} = 0 \tag{A14}
\]
FIGURE A2 SCHEMATIC CONE AND PLATE VISCOMETER
And now for constant shear stress

\[ \tau \Theta \phi = \mu a \sin \Theta \frac{d\Omega}{d\Theta} = \frac{d\Omega}{d\Theta} \quad (A15) \]

where \( \mu a \) is the apparent viscosity. Integration of Equation A15 subject to the boundary conditions

\[ \begin{align*}
\Omega &= 0 \quad \text{at} \quad \Theta = \frac{\pi}{2} + \Theta V \\
\Omega &= \Omega_0 \quad \text{at} \quad \Theta = \frac{\pi}{2}
\end{align*} \]

gives

\[ \frac{\Omega}{\Omega_0} = \frac{\pi/2}{\Theta C} - \Theta \quad (A16) \]

This says that at constant radius the angular velocity varies linearly with angular position \( \Theta \). Hence, the shear rate is found to be

\[ \frac{d\Omega}{d\Theta} = -\frac{\Omega_0}{\Theta C} \quad (A17) \]

Equations A7 and A17 provide the design criteria for cup and bob combinations. That is, for a given radius and gap the necessary angle can be determined by combining Equations A7 and A17 to give

\[ \frac{1}{\Theta C} = \frac{K^2}{1-K^2} \quad (A18) \]
APPENDIX B

THE CAPILLARY VISCOMETER

The capillary viscometer is in reality a case of tube flow.

In this simple case of a "very long" tube, laminar flow, constant density, and no end effects, a momentum balance yields the equation

$$\lim_{\Delta r \to 0} \left[ \frac{(r \tau rz) lr + \Delta r - (r \tau rz) lr}{\Delta r} \right] = \left( \frac{P_0 - P_L}{L} + pg \right) r$$

which in the limit is

$$\frac{d}{dt} (r \tau rz) = \left( \frac{P_0 - P_L}{L} + pg \right) r \quad (B1)$$

This can be integrated and the constant of integration evaluated by the condition that the momentum flux must be finite at \( r = 0 \)

$$\tau L^2 = \left( \frac{P_0 - P_L}{2L} + pg \right) r \quad (B3)$$

It will be observed that the shear stress at the wall is

$$\tau W = \frac{\Delta P}{2L} R \quad (B4)$$

By using an expression for Newton's law of viscosity, the shearing rate can be expressed as

$$\dot{\gamma} = \frac{\Delta P}{2 \mu L} r \quad (B5)$$

or

$$\dot{\gamma} W = \frac{\Delta P}{2 \mu L} R \quad (B6)$$

To relieve the requirements for no end effect, the technique of Hagenbach-Covette can be used. The basis for the correction is to compare two capillaries that differ only in length and compare the
pressure needed to obtain the same flow rate from both. If the height of blood is the same in both reservoirs, the corrected pressure gradient is

\[ \widehat{\Delta p} = \frac{P_B - P_A}{L_B - L_A} \tag{B7} \]

where the subscripts denote the different capillaries.

This correction is then used in Equation B4 to give the true wall shear stress as

\[ \tau_w = \frac{R}{2} \widehat{\Delta p} \tag{B8} \]

The true wall shearing rate can more correctly be found using the Rabinowitsch equation

\[ \dot{\gamma}_w = \frac{3Q}{\pi R^3} + \frac{Q}{\pi R^3} \left( \frac{d \log (4Q/\pi R^3)}{d \log (\hat{\rho} R/2)} \right) \tag{B9} \]

The true apparent viscosity is then

\[ \mu = \frac{\widehat{\tau}_w}{\dot{\gamma}_w} \tag{B10} \]
APPENDIX C

Plasma Hemoglobin Determination

This technique for the determination of plasma hemoglobin is based on the reaction of hemoglobin with benzidine to produce a color shift reaction.

The essential difference in this particular technique as compared to other benzidine methods is the use of 3x crystallized benzidine rather than the less pure commercial form. This results in increased sensitivity and reproducibility.

The procedure used in this laboratory uses the reagents:

a. .7% Benzidine in glacial acetic acid
b. 3% Hydrogen Peroxide
c. Isotonic Saline

Standards for the analysis are provided by diluting lysised whole blood, which has been assayed by the cyanmethemoglobin method, to known concentrations with saline.

If the samples to be analyzed have a high concentration as evidenced by the marked red color they are diluted to lower levels with saline.

The procedure for the analysis of the diluted samples and plasma sample is then to mix .4 ml. of sample with 6 ml. of
benzidine reagent. 0.2 ml. of hydrogen peroxide is then added and mixed with previous step. As this reaction depends on a non-stable color shift, the sample is immediately transferred to a cuvette and the optical density at 700 m is followed until the maximum value is obtained. This value is then compared to the values obtained from the standards and by multiplying by the dilution factor the concentration of hemoglobin can be obtained.