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THE EFFECTS OF SHEAR STRESS ON THE CLOTTABILITY OF FIBRINOGEN IN PLASMA

by

BRUCE C. PETERS

A THESIS SUBMITTED
IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

Thesis Director's Signature:

Houston, Texas

April 1975

ABSTRACT

The Effects of Shear Stress On The Clottability
Of Fibrinogen in Plasma

by

Bruce C. Peters

One problem encountered in the use of circulatory-assist devices has been alterations of the coagulation mechanism. This study was made to determine if one source of this coagulation problem could be due to increased shear stress in the extracorporeal systems.

A plasma pool was collected from thirteen donors and lyophilized to provide identical plasma samples for all experimental results. Tests were made with various shear stress and shear exposure times to determine the effects of these variables. A constant shear stress viscometer was used in this examination, and two systems with different surface to volume ratios were available. The surfaces were siliconized before the plasma was introduced to the viscometer. To determine the magnitude of any surface losses, tests were made with radioactive Iodine (I^{125}) labeled fibrinogen.

The tests with the I^{125} labeled fibrinogen showed that no loss of fibrinogen occurred due to adhesion to the surface. Studies made with constant time exposures and varying shear stress indicated that clottability does decrease with increased shear stress up to 1000 dynes/cm^2, at which point an apparent minimum clottability occurred. This loss was attributed to conformational changes causing steric interferences with polymerization sites. The minimum was considered to be caused by a maximum in the steric hindrance of the polymerization sites due to conformational changes.

Studies done at constant shear stress, varying time indicate that loss of clottability is a function of both exposure time and shear stress. Variation of the surface to volume ratio suggested that the clottability loss is probably surface dependant.

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ACKNOWLEDGEMENTS

I would to express my appreciation of those who provided both time and guidance in making this study:

Dr. J. David Hellums, my thesis advisor,

Mr. L. Byron Leverett, who helped with many problems,

Dr. L. V. McIntire, who provided much guidance during Dr. Hellums absence and served as chairman of the oral examination committee,

Mrs. Denise Smith and Miss Marcella Estrella, who did the necessary chemical analyses,

Drs. C. D. Armeniades and C. P. Alfrey, Jr. who also served on the oral examination committee, and

my friends in Houston that kept life enjoyable.

NOMENCLATURE

c-c	cone and cone
c-p	cone and plate
cssv	constant shear stress viscometer
f	frictional resistence/unit length of molecule
G	velocity gradient
I.D.	inside diameter
k	bob O.D./cup I.D.
L	molecule length
O.D.	outside diameter
R	radius
rpm	revolutions per minute
V_{rpm}	digital voltmeter reading for rpm
V_{temp}	digital voltmeter reading for temperature
V_{torq}	digital voltmeter reading for torque
η, μ	viscosity
ν	kinematic viscosity
δ	molecule diameter
τ_{max}	stretching force
γ	shear rate

INTRODUCTION

One problem encountered in the use of some circulatory-assist devices is the inability to maintain normal coagulation characteristics in the blood. The first step in the correction of this problem lies in the study of the effects of extracorporeal flow on the various components of the blood. Most work done thus far at Rice University with blood has been concerned with the study of the formed elements, namely red blood cells and platelets. The work presented here follows the shear studies done previously at Rice with red blood cells and platelets,[23,25] but is concerned with the effects of shear stress on fibrinogen, a plasma protein, rather than stress effects on the formed elements of blood.

Three main areas of interest are dealt with in this study:

1) Determination of the amount of fibrinogen lost to the surface of the equipment,
2) Relationship between fibrinogen destruction and time at a constant shear stress, and
3) Relationship between fibrinogen destruction and shear stress for constant time exposures.

The shear stresses used for these studies ranged from 0 to 3300 dynes/cm^2 and the shear times from 0 to 70 minutes.

BACKGROUND ON FIBRINOGEN

Fibrinogen (Factor I) is the basic plasma protein used in the coagulation mechanism of blood. It acts as a monomer which will polymerize to form fibrin in the presence of thrombin. This fibrin will cross-link in the presence of Factor XIII (the fibrin stabilizing factor) to form the insoluble clot.

The fibrinogen molecule is an elongated molecule with a molecular weight of 340,000. It is thought to be a dimer made from two identical halves, each half consisting of three polypeptide chains (The A (α) chain, the B (β), and the γ chain) which are held together by disulfide bridges with the two halves bonded with disulfide bonds. Sedimentation techniques have shown the molecular weights of the polypeptide chains to be 63,500 for the A (α) chain, 56,000 for the B (β) chain, and 47,000 for the gamma (γ) chain.[3]

There are possibly three different types of fibrinogen molecules in plasma. The first type is a low solubility molecule which complexes with cold-insoluble globulins. This molecule has just recently been isolated, and little is known of its structure. A molecule of intermediate solubility is the second type. These molecules show a different chromatographic behavior, and have differences

in the H_2 N-terminal end of the molecule. The third type is a high solubility molecule with a lower molecular weight than the intermediate type, and is possibly produced by proteolysis of the intermediate type by plasmin. This plasmin has been shown to cause a degradation of both the A (α) and B (β) chains. Most of the antibodies that act on fibrinogen are monospecific towards the various chains, and since the γ -antibodies do not act on the fibrinogen molecules, this indicates that the alpha and beta chains are on the surface of the molecule[2].

Different types of the same polypeptide chain in pooled plasma have been found. At least three different alpha chains with identical amino-terminal ends, but with varying electrophoretic mobility and molecular weights exist. In bovine fibrinogen, which is similar to human fibrinogen, at least two different beta and gamma chains have also been found[2].

A number of possible reasons could account for the variances in the chains. Proteolysis by endo- and exo-peptidases, by amidases, or by phosphatases could shorten the chains. Varying availability of carbohydrates and phosphorous used in the bio-synthesis or fibrinogen could produce differences in the chains. Also genetic differences could be a cause of the variances.

Fibrinogen will form the ordered structure or fibrin once the acidic peptides at the amino-terminal ends of the alpha and beta chains have been removed. Studies have shown that the alpha fibrinopeptides are released first and begin polymerizing, then after a certain lag-time the beta fibrinopeptides are released and also begin polymerizing. It is assumed that the conformational changes in the molecule are the source of this lag time. Once the alpha chains are activated, they begin to unwind with polymerization, thus allowing room for the beta chains to polymerize. Studies with reptilase have shown that it releases only the alpha chain and causes more end to end bonding than thrombin. However, polymerization does still occur. This would indicate that two bonding systems occur simultaneously, one with the alpha chains and one with the beta chains. It is thought that the alpha chains are joined at 120^{o} angles, and the beta chains at 60^{o} angles, thus making every third alpha chain parallel and every sixth beta-chain parallel.[2]

Because of the number of differences that can be found in different fibrinogen molecules, and the size of the molecule, further detail on the structure of fibrinogen could not be fully covered here. However, much has been written on the molecule's structure, its part in the clotting mechanism in the human system, and factors other

than shear which can affect its clotting mechanism.

Some of these can be found in the references.[2,3,14,19,20,21,22,29,30,32]

PREVIOUS WORK ON SHEAR OF BIO-MACROMOLECULES

Although it has been known for some time that cleavage of large biomacromoles could be caused by shearing forces, very little work has been done thus far in the study of the effects of shear on fibrinogen. To the knowledge of this author, only one study, that of Charm and Wong[5], has been documented. Work has been done in this field with other bio-macromolecules, notably deoxyribonucleic acid (DNA)[24], and enzymes[6].

In their study, Charm and Wong used two independent systems to demonstrate a loss in clottability of fibrinogen in plasma with shear. Plasma was sheared in a Weissenberg Rheogoniometer at various shear rates between 290 sec^{-1} and 1155 sec^{-1} for varying times at two temperatures (4.0°C and 37°C). Also, plasma was recycled with a finger pump through a cylindrical tube at known flow rates for varying times to demonstrate clottability loss due to shear in this system. The mass average shear was calculated for the tube system[6], and clottabilities were experimentally determined for different shears. Their results showed fairly close agreement between the two systems. No appreciable effects of temperature were observed. It was found that at constant shear rates increased exposure

time produced a decrease in the amount of clottable fibrinogen left in the system. When the product of shear rate times time was plotted against loss in clottability, data for all shear rates (at both temperatures) produced a fairly smooth curve. Using this curve and assuming a mass average shear per second of 1470 sec^{-1} in the body, they determined that a 50% loss in clottability (or half-life) would require approximately four days in vivo. Other studies have shown that the in vivo half-life is about four to five days, which would indicate the in vivo loss could be almost entirely due to shear. However, they did assume no loss to the surfaces, and used rather low shear rates over long periods of time (up to five days)[5]. A gradual surface build-up of fibrinogen or proteolysis by other plasma components could have accounted for some loss of clottability.

The assumption of no surface loss was based on previous work they (Charm and Wong) did in their study of inactivation of various enzymes with shear[6]. Using a similar procedure to that used with fibrinogen they obtained similar results, i.e. loss of enzyme activity with increased mass average shear or dimensionless shear rate (shear rate x time). Although not mentioned, it can be assumed that surface effects on the enzymes were considered, and found to be negligible[5]. Comparison of Charm and Wong's results with the results of this study can be found in the Discussion of Results.

As early as 1944, Frenkel demonstrated the existence of a critical velocity gradient in a fluid containing macromolecules, above which rupture of the molecules would occur.[13] At lower values alignment of sections of the molecules with the stream lines will occur, while at higher values extending forces due to the gradient will be greater than the bond strengths and cause splitting of the molecule.

Levinthal and Davison applied this work to their investigation of the degradation of DNA molecules by shear[24]. Using a capillary tube approximately twice the molecule length in diameter, they calculated the tensile force a rod of similar dimensions to the DNA molecule would be exposed to as a function of velocity, and showed that these forces were greater than the strengths of some of the covalent bonds present in the molecule. Experimental results were in agreement (within one order of magnitude).

VISCOMETER

The equipment used in this study was a constant shear stress viscometer (CSSV) similar to that used by R. N. MacCallum and R. F. Lemuth in their studies with red blood cells and platelets.[23,25] It was designed by W. Ruska, and machined by Mr. Hugh Hales, Mechanical Engineering Machine Shop, Rice University. Design is such that a constant shear stress is expected throughout the fluid volume when in laminar flow.

The basic design consists of a rotating outer cup revolving about a stationary inner bob, aligned in such a way as to give a concentric cylinder configuration. The bottom of the cup is flat, while the bottom of the bob is machined with a slight angle to give a cone-and-plate configuration when in place. This cup top is attached to the drive shaft to maintain alignment and machined in the form of an inverted cone with the sides 60^{o} off the axis. When matched against the conical section of the bob, this gives a cone-and-cone viscometer region at the top. Figures 1 and 2 illustrate this design. Since the theoretical basis for this design has been previously considered it will not be covered here.[25a]

Table 1

Cup and Bob Data

Bob	I	II	III
Cup I.D. (cm)	6.9985	6.9985	6.9985
Bob O.D. (cm)	6.9713	6.9507	6.9220
Gap width (cm)	0.0136	0.0239	0.0383
K (Bob O.D./cup I.D.)	0.99612	0.99318	0.98908
θave	59.8°	59.67°	59.5°
θc-p	0.25°	0.4°	0.667°
θc-c	0.2°	0.33°	0.5°
Volume c-p section (cc)	0.39	0.62	1.04
Volume cyl. section (cc)	2.09	3.67	5.86
Volume c-c section (cc)	0.21	0.36	0.58
Total sheared volume (cc)	2.7	4.7	7.5
Surface/Volume (cm^{-1})	176.8	100.5	62.7
γ/rpm (sec^{-1}/rpm)	26.92	15.29	9.54
R_1 (cm)	.9525	.9525	.9525
R_2 (cm)	1.27	1.27	1.27
Top gap (cm)	0.0059	0.0099	0.0148
Bottom gap (cm)	0.0017	0.0028	0.0047

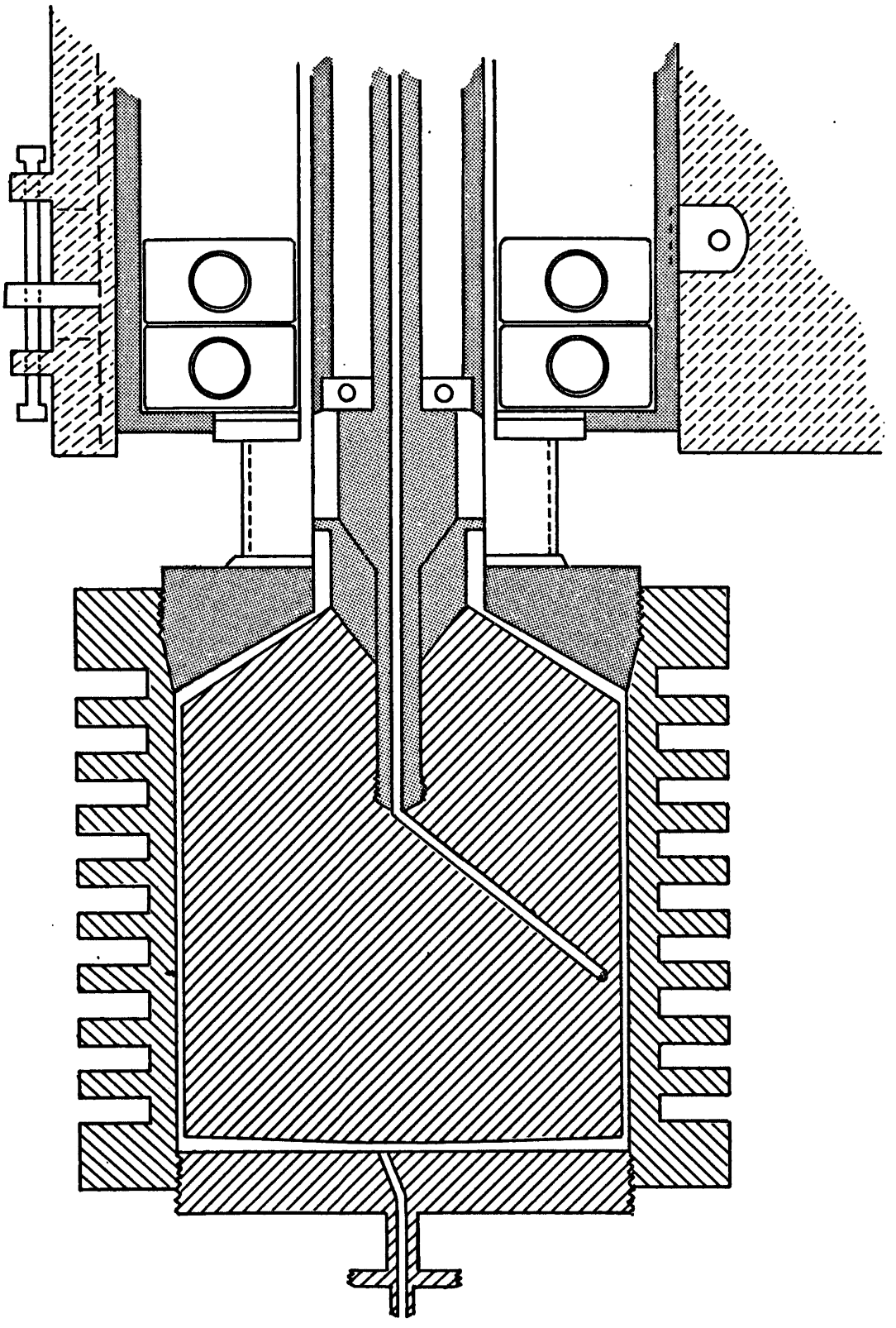


Figure 1

The Constant Shear Stress Viscometer (CSSV)

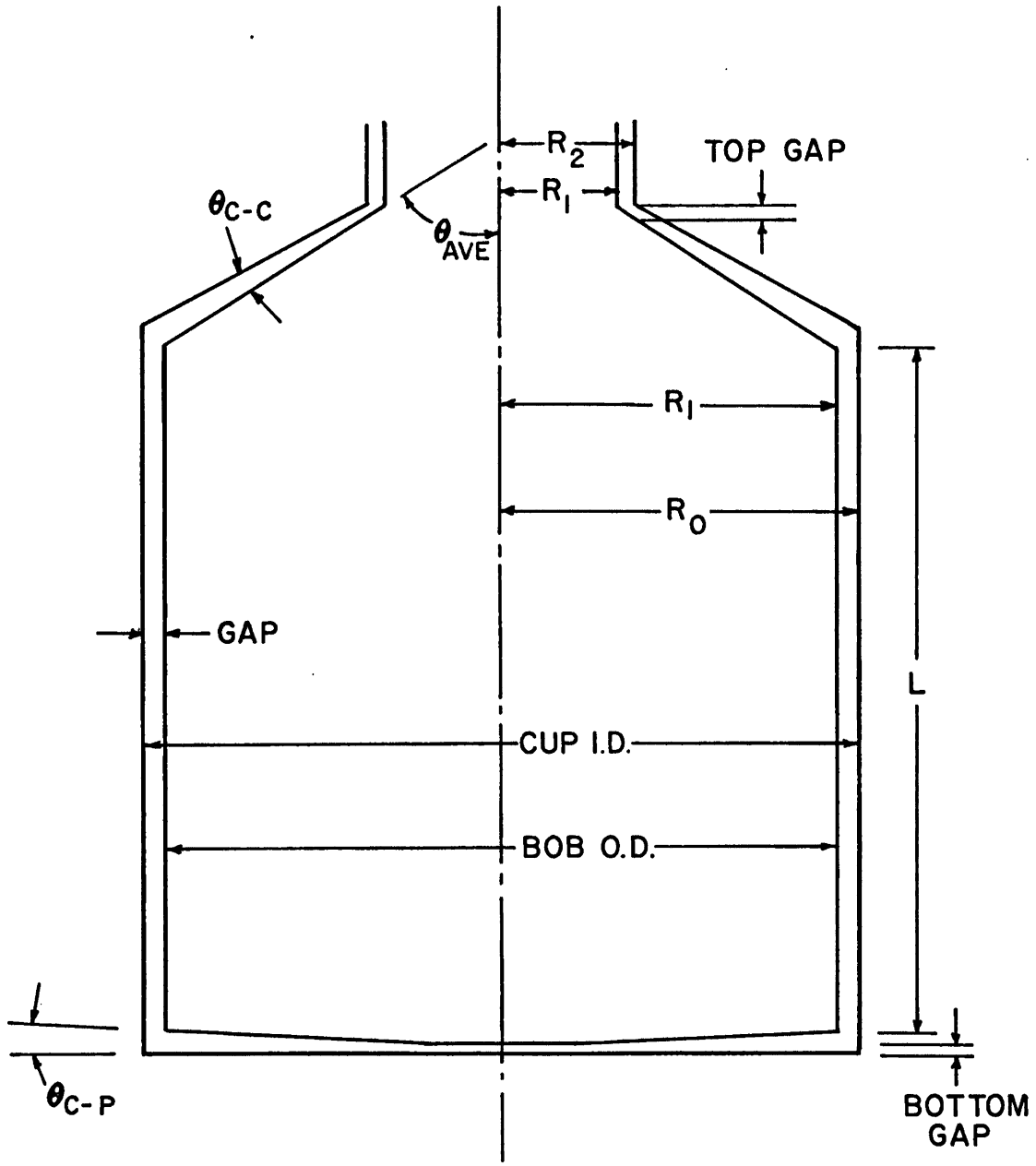


Figure 2

Constant Shear Stress Viscometer[23a]

Three variables on this viscometer could be measured with the instrumentation used with it: temperature, cup rpm, and bob torque. These were monitored using three analog digital voltmeters attached to the control unit. The outputs of these three voltmeters (henceforth known as V_{temp}, V_{torq}, and V_{rpm}) were calibrated such that they could easily be converted to the desired units of degrees centigrade (V_{temp}), dynes per square centimeter (V_{torq}), and actual rpm (V_{rpm}).

The temperature was monitored by placing an iron-constantan thermocouple approximately 0.1 inches inside the surface of the bob. This thermocouple was calibrated using an ice water bath (0°C) and a warm water bath of known temperature (found using a standard mercury thermometer), and was determined to be within 0.5°C agreement with standard emf °C tables for iron-constantan thermocouples.

The cup rpm was calibrated using a Jaquet's indicator (product of H. H. Sticht Co., Inc., New York, New York). Approximately five readings were taken at each point, and the average of these used as the actual rpm. Twenty-two points were taken in the range of 0 → 4300 rpm, and plotted to determine the conversion factor from millivolts to rpm. Both calibration data and graph can be found in Appendix I.

A torsional spring is attached to the bob shaft to hold it in place. The shaft is also attached to a Gould

transducer, Model 761803993, which measures the amount of deflection of the bob due to the torque exerted on it by the fluid. The transducer output is monitored by the analogic digital voltmeter. This output (V_{torq}) was first calibrated using "dead weight" measurements to obtain the calculated shear stress necessary to obtain a given deflection.[25d] Next, an experimental calibration was made using a standard silicon oil of known viscosity. Comparison of the shear stresses obtained by these two methods showed an agreement within three percent. Part of this error could be attributable to the amount of error in the machining of the cup and bob, and to error in temperature measurement, so the "dead weight" calibration was used. Data and calculations for this calibration can be found in Appendix I.

The cup used for all experiments was an aluminum (series 7075T6) cup, hard anodized to a maximum buildup of .0005 inches. The cup top was made of series 410 stainless steel, but most plasma touching this surface was not used in analyses. Three bobs were available for use, all aluminum, series 7075T6. Each of these was also hard anodized over the surfaces on which shear took place. These bobs varied in size so as to allow variation in the surface to volume ratio in the viscometer. Tolerances were held within .0005" on both the cup and the bobs. The bob lengths varied somewhat but all were

2.760 $\pm$ .001 inches in length. Table 1 gives the angles and equipment sizes used in each configuration. Only two bobs (designated bobs I and II in Table 1) were used in this study. The low turbulent transition point disallowed the use of bob III at the shear stresses under consideration. Also temperature problems become more significant with the bob III configuration.

Concentricity of the cup and bob was measured periodically using a dial indicator attached to the top cone (drive shaft) and rotated about the bob in use. In all cases the total deflection of the indicator was less than .0005", thus giving a radial error of less than .0003", much of which could be due to machining error.

Assembly of the viscometer consisted of screwing the bob onto the bob shaft, setting the cone and cone gap using a Starrett gauge, Model 25-481 for measurement, attaching the cup to the shaft cone and setting the bottom gap using another model 25-481 Starrett gauge which had been modified for use as a depth gauge.

During the series where high shear stresses were attained, a flow transition was observed. At lower rpms, the ratio V_{torq}/V_{rpm} was nearly constant (there was some decrease in this ratio as the rpm was increased due to viscous heating), while at rpms above this point the ratio would increase. Both MacCallum and Lemuth observed similar action in their work, and MacCallum referred to

this as the "turbulent transition". Using MacCallum's method, the theoretical transition to turbulence should occur at 6200 rpm with bob I and at 3550 rpm with bob II.[25c] However, tests done with plasma show that the apparent viscosity will increase at much lower values, ≃2700 rpm for bob I and ≃2100 rpm for bob II. MacCallum attributed this to secondary flows occurring in the conical sections of the viscometer. M. Fewell's work with secondary flows in cone-and-plate viscometers also would support this reason.[12]

EXPERIMENTAL PROCEDURES

Plasma Pool

Fibrinogen levels vary greatly from person to person, along with the concentrations of other plasma components. In order to have a consistent plasma for the data runs, a plasma pool was collected in sufficient quantity for all runs, and lyophilized. Approximately three liters of plasma were collected from thirteen donors. Of these donors, six were women, and three smoked cigarettes. All were between the ages of twenty and forty. The plasma was collected early in the morning, before the donors had eaten that day. All were in good health, and none were on medication (including aspirin) at the time of collection.

To avoid handling problems, four or five donors were used each of three different dates to form three separate pools, thus necessitating the handling of only 1200 ml to 1300 ml of plasma at a time.

The blood was collected in 500 ml PliaPak^{R} (Product of Abbott Labs) bags containing 50 ml of 3.2% (wt.) solution of sodium citrate. Approximately 450 ml of blood was collected in each bag. The full bags were centrifuged at 3000g for 15 minutes to give platelet poor plasma (PPP),

and this plasma was pooled in a 2000 ml beaker. HEPE's Buffer was added in sufficient quantity to give a 1% by weight solution, and gently stirred until dissolved. This pooled plasma was then placed in 20 ml serum bottles in 10 ml aliquots using a graduated 10 ml pipet, and immediately frozen and lyophilized using the equipment in the Hematology division at Methodist Hospital, Houston, Texas.

At the time of use for data, equivalent amounts of each pool were diluted, dissolved, and pooled to give a common pool from which samples were taken. The plasma was diluted by adding 10 ml of distilled water to each of the lyopholized 10 ml aliquots and letting sit at room temperature (18-22^{o}C) for thirty minutes. After the lyopholized plasma was completely dissolved, it was poured into a common beaker and covered until needed. The time involved between preparation and analysis was usually:

1) Approximately one hour total for preparation,
2) Five to seven hours for shearing procedure,
3) One-half hour for transportation to Methodist Hospital for analysis, and
4) Two-three hours for analysis,

thus taking less than twelve hours from the start of the plasma preparation to the finish of the plasma thrombin time and total fibrinogen analyses.

In some of the earlier series (5-8) the diluted plasma was refrigerated during the shear runs. However, since some tests showed that refrigeration or freezing might have some effect on the clottable fibrinogen, this procedure was discontinued (see Appendix II).

Cleaning Procedure

Before any data was taken using plasma, all surfaces were cleaned with a 50/50 solution of toluene and acetone to remove any silicone oil left from the calibration work. The bobs and the cup parts were placed in an Mettler Electronics ultrasonic cleaner (Model ME 1.5) containing the toulene/acetone mixture for one hour. These parts were then washed with tap water and Alconox^{R} detergent, rinsed with tap water, then rinsed with distilled water. These parts were then coated with Siliclad^{R} (a Clay-Adams product) according to the manufacturer's specifications for coating metals to obtain siliconized surfaces on these pieces.

The top cone portion of the cone and cone section (henceforth referred to as the shaft cone) was soaked in a similar toulene/acetone solution, washed with tap water and Alconox^{R} detergent, rinsed with tap water, rinsed with distilled water, then rinsed with saline solution (0.9% sodium chloride injection, U.S.P produced by Baxter, a division of Travenol Laboratories, Inc.). This was the only surface not coated with Siliclad^{R}.

This procedure was used only when silicone oils had contacted the surfaces since the last cleaning. Before each series of data was taken, the bobs and cup were washed with tap water and Alconox^{R} detergent, rinsed with tap water and distilled water, then coated with Siliclad. The shaft cone was washed and wiped clean using Micro-wipes, a product of Scott Paper Company, then rinsed with distilled water. The washing procedure used before each trial of a series consisted of rinsing with tap water, then rinsing with saline both cup and bob. These were then dried using microwipes in the laboratory.

The shaft cone was cleaned by wiping repeatedly (three times) with Micro-wipes soaked with distilled water, then rinsing with distilled water. This surface was not rinsed with saline in order to minimize the damage to the equipment (saline caused oxidation of the shaft in the dead space above the cone).

Shear Procedure

Once the plasma had been prepared, and the equipment had been cleaned, siliconized, assembled, and aligned, the cup was loosened from the shaft approximately 0.5 cm and the plasma was placed in the viscometer through the bottom drain valve with a Plastipak disposable syringe (5 cc syringe for bob I, 10 cc syringe for bob II) connected to the drain valve with a 5-7 cm piece of tubing cut from

a Venoset^{R} (product of Abbott Labs). Air was removed from the syringe and tube before connection to the valve. Once the valve had been closed (with syringe and tube still attached) the cup was slowly screwed back into position on the shaft cone. This method served two purposes:

1) By allowing a large volume at the bottom while the plasma was flowing into the viscometer the chances of causing damage to the plasma by hitting the bottom of the bob at a high velocity are diminished, and
2) The chances of air entrapment in the sheared volume are lessened.

The timing of the shear runs was done with a stopwatch, marked in increments of 0.2 seconds. It was started simultaneously with the start of the cup motion, and stopped when the cup drive was shut off. Generally, about fifteen to twenty-five seconds were required to adjust the rpm to the proper setting, and about five to seven seconds were needed for the cup to coast to a stop after the drive had been shut off.

The control unit allowed manual control of only the cup rpm. Thus the cup rpm had to be manually increased until the desired torque (shear stress) reading was attained. Normally, the rpm had to be increased during

the time of the run to maintain a constant torque reading because of viscous heating, which would cause a decrease in the plasma viscosity. This was a source of error in determining the shear stress of a given run, since the only way to know when to increase the cup rpm to cancel the effect of viscosity decrease was to observe when the torque reading (V_{torq}) changed. For the systems used, a change of one millivolt in V_{torq} was the equivalent of a 23 dynes/cm^2 change in the actual shear stress. Fortunately, at the lower shear stresses where this would be most significant, the viscous heating was much less and would reach a point where cooling due to the motion of the finned cup would be equivalent to the heat generated, allowing much easier control of the shear stress. At the high shear stresses (above 1500 dynes/cm^2) compressed air was blown on the cup to provide enough cooling to keep the fluid temperature below 37^{o}C. This type of cooling procedure caused fluctuations in the viscosity of the plasma, thus causing fluctuations in the shear stress. The values of V_{torq} in the raw data for these high shear stresses are averages of the ranges of V_{torq} observed during a given run.

Various tests were made on the plasma once it had been sheared. Because of this, the plasma volume needed exceeded the amount of plasma sheared in any section of

the viscometer. Once a run had been completed, the cup was loosened to the filling position and the plasma from the concentric cylinder section allowed to drain into the cup bottom and mix with the plasma sheared in the cone and plate section. The material in the cone and cone section remained there due to capillary action. After allowing two to three minutes for draining, the cup was removed and the plasma removed with another disposable syringe and tube, and placed in 12 x 75 mm tubes (No. 2003, made by Falcon, a division of Becton Dickinson & Co.). Approximately 2.50 cc of plasma could be recovered with the bob I configuration and 4.5 cc with the bob II configuration. After removal of plasma the equipment was cleaned in the described manner.

Analysis

For each series of data, two control samples were taken: one from the pool before any shear runs were made, and one after all shear runs had been completed. Comparison of these two could show if the initial control was completely dissolved, or if the glass container had any effect on the clottability of the plasma. Initially, tests on the sheared plasma included a Tyrosine Test (a modification of Ratnoff-Menzie technique) for total fibrinogen, Thrombin Times (TT's) from which clottable fibrinogen could be determined, Prothrombin Times (PT's), and

Partial Thromboplastin Times (PTT's), and electrophoresis polyacrylamide gels of the clots formed were also made with samples of some of the series. Little change was seen in the tyrosine test (note Series 2,3) while significant changes could be found in the thrombin time peaks. Since the PT's and PTT's were not concerned directly with fibrinogen clottability, they, along with tyrosine tests, were discontinued to allow multiple thrombin time tests. All tests (Series 4-22) except the electrophoresis gels were performed by Mrs. Denise Smith, technician in the Hematology Division of Methodist Hospital, Houston, Texas. Miss Marcella Estrella, Technician, Biomedical Engineering Department, Rice University, Houston, Texas, performed the tests on Series 1-3 and other tests (see Appendix II).

A Bio-Data instrument, product of Bio-Data, Hatboro, Pennsylvania was used to perform the TT's, PTT's, and PT's, according to instrument instructions. The procedure used in making the tyrosine test can be found in Appendix II.

DISCUSSION OF RESULTS

As mentioned previously, the efforts of this study were aimed at three goals:

1) to determine the effects of and loss to the surfaces of the equipment (CSSV);
2) To determine the effects on clottability of a constant shear stress at varying exposure times, and;
3) To determine the effects on clottability of various shear stresses for equal time exposure to shear.

Both the reduced data and raw data used in these studies can be found in Appendices III and IV respectively.

Surface Effects

Chromium (Cr^{51}) Study

To determine the degree of fibrinogen loss to the surfaces of the viscometer, radioactive isotope techniques were used. First, radioactive chromium (Cr^{51}) was used in the plasma to determine the quality of the cleaning procedure. Since Cr^{51} does not label any particular plasma component, and is present only in a very dilute concentration, it should give a good analysis of the cleaning procedure.

A pool of plasma was made from the lyophilized samples, and this pool split into two smaller pools, designated pools "A" and "B". To pool "A" a small amount of Cr^{51} was added and mixed thoroughly. A sample of each pool was then taken to obtain a control radioactivity count for each pool. These counts can be found in Table 2 (samples A1 and B1).

Next, a sample of pool "A" was placed in the viscometer, the outer cylinder was spun by hand a few times, then the sample was removed and a count was taken (A2). After cleaning the equipment in the described manner, a sample of pool "B" was placed in the viscometer, spun, removed, and counted in a like fashion (B2).

After again cleaning, a sample of pool "A" was placed in the viscometer, sheared at a shear stress of 1370 dynes/cm^2 for fifteen minutes, and then counted (A3). A sample of pool "B" was then treated in the same manner (B3), again after cleaning the equipment. Finally, this procedure was repeated with both pools "A" and "B" at a shear stress of 2773 dynes/cm^2 instead of 1370 dynes/cm^2 (A4 and B4).

As can be seen from Table 2, the counts of the "clean" pool samples (pool "B") increased with each successive run. However, there was no large amount of loss in the pool "A" samples, indicating that the surface losses are fairly small in comparison to the fluid

concentration of Cr^{51} (and ions of similar size). The increase in the successive counts of the pool "B" samples could be due to a gradual build-up on the surfaces of the equipment, but even if the residual material left on the equipment after cleaning was due only to the preceding pool "A" run, the total change in concentration would be a maximum of two percent (above a shear stress of 2773 dynes/cm^2), which would not account for the changes in clottability noted during the shear runs (discussed later).

Iodine (I^{125}) Study

To test if larger components like fibrinogen are lost to the surfaces a different test is needed. This was accomplished by adding a small amount of radioactive iodine (I^{125}) labeled fibrinogen to a reconstituted pool of lyophilized plasma, shearing samples from this pool at various shear stresses, and determining any changes in radioactivity for the various samples. A noticeable decrease in radioactivity for any sample would indicate a loss of fibrinogen.

The results of this study can be found in Table 3. As can be seen from the data in Table 3, there was virtually no change in fibrinogen concentration for any of the samples, and statistical analysis (using the method of Johnson and Leone[9]) based on the control samples shows that all samples had statistically the same fibrinogen concentration after shearing.

TABLE 2

Sample	Shear Stress (dynes/cm^2)	Time (Mins)	Count	Comments
A1	0		304,001	control
B1	0		≈0	control
A2	≈1	1	292,739	in CSSV, handspun
B2	≈1	1	1,368	in CSSV, handspun
A3	1370	15	301,110	
B3	1370	15	3,217	
A4	2773	15	292,748	
B4	2773	15	4,779	

From the data obtained with the Cr^{51} and I^{125} studies it can be assumed that no significant loss of plasma material to the equipment surfaces occurred. Repitition of these tests with a different surface to volume ratio was assumed unnecessary in view of these results.

Plasma Analysis

Refrigeration Effects

In the early series (Series 1-3, see Table 4), only tyrosine tests were made on the collected samples, and comparison of the clottabilities at the various shear stresses showed that there was essentially no change in the clottabilities of these samples. These samples had been frozen before testing and a test was made to determine the effects of freezing and refrigeration on the lyophilized plasma.

A large pool was made from which twenty-four separate samples were taken. Eight of these samples were analyzed immediately (with the tyrosine test) to give a control clottability. Of the remaining sixteen samples, eight were placed in a refrigerator at approximately $5^{o}C$, and eight were placed in a freezer at approximately $-18^{o}C$, and both groups were kept there for two days before analyzing (again analysis by the tyrosine test). Table 5 shows the results of this study.

TABLE 3A

Series 9 (Bob I)

I^{125} Labeled Fibrinogen — 20 Mins/Sample — 2 Tests/Sample

Sample	Shear Stress (dynes/cm^2)	Analyzed Vol_1 (ml)	$Count_1$	Analyzed Vol_2 (ml)	$Count_2$	Comments
1	0	0.9	338297	0.9	348695	Control
2	0	0.9	327005	0.9	332560	Control
3	495	0.9	320004	0.9	333930	
4	1000	0.9	324795	0.9	331295	
5	1508	0.9	330635	0.9	332305	
6	2037	0.9	346835	0.9	335505	
7	2520	0.9	347098	0.9	356709	

TABLE 3B

Series 10 (Bob I)

I^{125} Labeled Fibrinogen 20 mins/sample

Sample	Shear Stress	Analyzed Vol_1	$Count_1$	Analyzed Vol_2	$Count_2$	Comments
1	0	0.9	331597	0.3	116627	Control
2	472	0.9	330439	0.9	349340	
3	1023	0.9	315903	0.9	333876	
4	1505	0.9	344506	0.9	323812	
5	2034	0.9	335857	0.9	344843	
6	2492	0.9	350648	0.9	352349	
7	0	0.9	354583	0.9	326254	Control
8	0	0.9	000315	0.9	000881	Blank

Both series 9 and series 10 samples came from the same plasma pool. The points 315903 (series 10, no. 4) and 356709 (series 9, no. 7) can be proven statistically acceptable using the method of Johnson and Leone. For 315903, the ratio $\frac{326254-315903}{354583-315903}(= .267)$ must be less than .512. For 356709, the ratio $\frac{356709-354583}{356709-326254}(=.069)$ is also below the critical value. Thus all counts can be considered the same.[18]

TABLE 4

MG% Fibrinogen From Tyrosine Test (App II)

Sample No.	Shear Stress (dynes/cm^2)	MG% Fibrinogen
	Series 1 (Bob II)	
1	0	210
2	23	185
3	519	205
4	≈2075	191
5	≈3100	205
	Series 2 (Bob II)	
1	0	147
2	519	164
3	1000	157
4	1760	176
5	2265	178
6	3256	157
7	2770	154
8	4475	176
9	0	161
	Series 3 (Bob I)	
1	3645	176 (20 min exposure)
2	0	180 (Control)
3	3670	197 (60 min exposure)
4	0	190 (in CSSV 15 mins, unsheared)
5	0	190 (control)

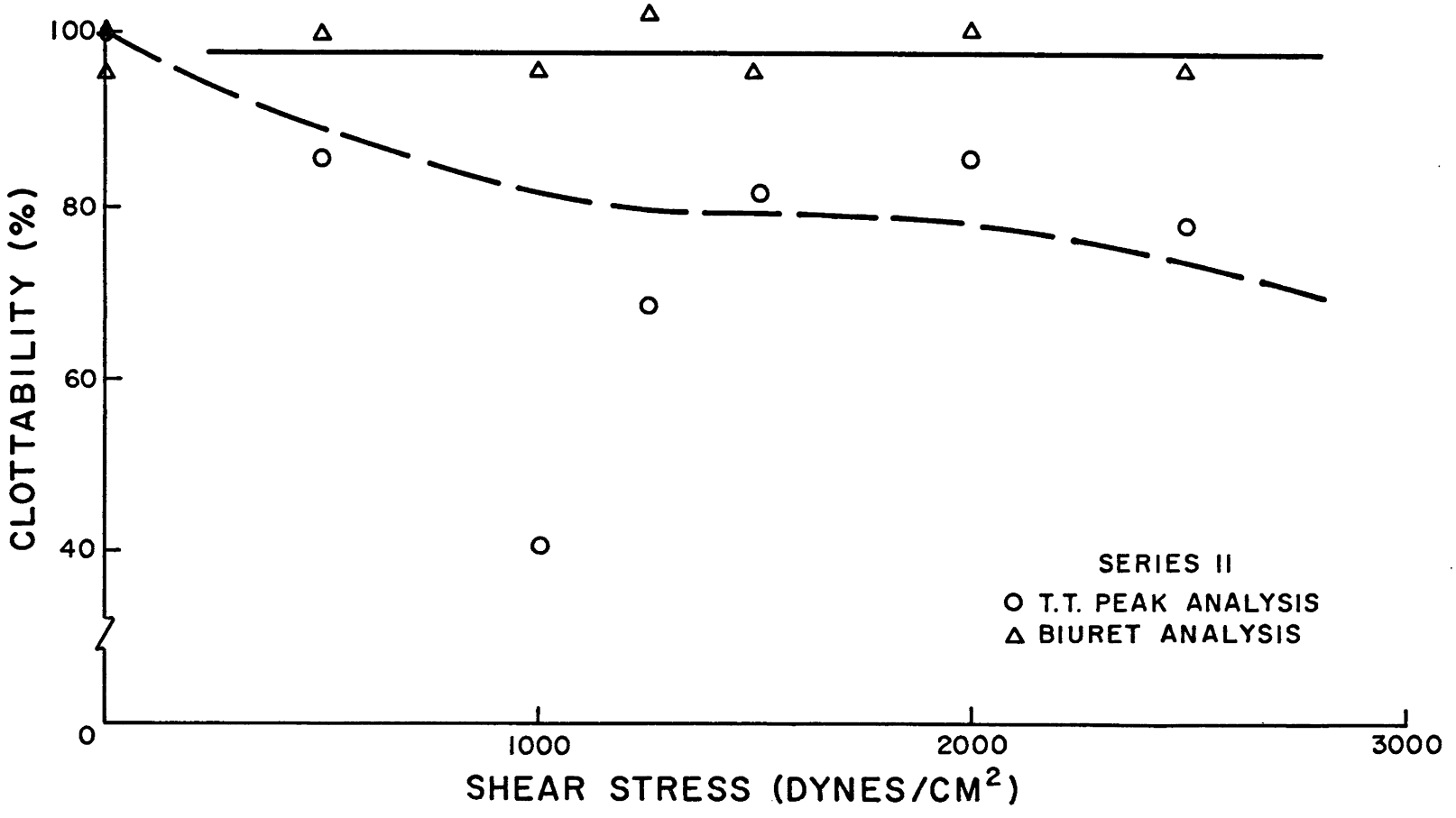


Figure 3

Comparison of Biuret Analysis and
Thrombin Time Peak Analysis

Although the tyrosine test is only accurate within ten percent, it can be seen from these results that there was an increase in clottability for both groups: about fifteen percent for the refrigerated samples and about ten percent for the frozen samples. These increases would indicate that the lyophilized plasma requires a fairly long time to dissolve in water (> 2-3 hours). This long term increase could tend to hide any small changes of the magnitude of the increase.

Thrombin Time Tests

Both the tyrosine test and the biuret test are tests for clottability which determine clottability by giving the concentrations of certain amino acid residues present in the clot. Thus, these tests are a measure only of the total protein in the clot, and do not measure the degree of polymerization. Also, as can be seen from the path, they are accurate only to ≈ 10 percent (Table 4).

The thrombin time tests used for most of the clottability analyses provide two types of information. It gives the actual thrombin time, and it also gives a measure of the extent of clotting from the peak values of the chart. The actual chart measurement is the rate of change of the optical density of the clot, and the peak value is a measure of the maximum optical density of the clot.

TABLE 5

Sample No.	MG% Fibrinogen 1st test	2nd test	Comments
1	133	164	Fresh plasma
2	147	145	Fresh plasma
3	138	152	Fresh plasma
4	145	159	Fresh plasma
5	147	145	Fresh plasma
6	159	143	Fresh plasma
7	161	145	Fresh plasma
8	138	145	Fresh plasma
9	185		Refrigerated 48 hours @ 5°C
10	173		Refrigerated 48 hours @ 5°C
11	183		Refrigerated 48 hours @ 5°C
12	187		Refrigerated 48 hours @ 5°C
13	164		Refrigerated 48 hours @ 5°C
14	168		Refrigerated 48 hours @ 5°C
15	192		Refrigerated 48 hours @ 5°C
16	183		Refrigerated 48 hours @ 5°C
17	161		Frozen 48 hours @ -18°C
18	187		Frozen 48 hours @ -18°C
19	150		Frozen 48 hours @ -18°C
20	176		Frozen 48 hours @ -18°C
21	164		Frozen 48 hours @ -18°C
22	164		Frozen 48 hours @ -18°C
23	145		Frozen 48 hours @ -18°C

The term "clottability" used in the following discussion of the shear stress and time versus percent clottability is actually a comparison of the changes in these peak values obtained with thrombin time tests. The reproducibility of these tests was about 5 percent, compared with the larger errors inherent in the tyrosine and biuret tests. As can also be seen from the results of series 1-3 (Table 4), the quality (protein content) of the plasma varied appreciably from day to day, so percent clottabilities were used rather than actual clottabilities, where the percent clottability is the ratio of the peak value (thrombin time test) for a particular sheared sample to the peak value of the controls obtained the same day from the same pool multiplied by one hundred per cent.

An example of the differences observed with the biuret test and the thrombin time peak analysis can be seen in series 11 (see Figure 3). Essentially no change could be observed with the biuret test, but a very definite change was noted in the thrombin time peak analysis. This was interpreted as an indication that the protein content of the clot is not affected by exposure to shear stress, but the clotting mechanism is altered by this exposure to shear stress.

Electrophoresis Gel Studies

The results of the electrophoresis gel studies (series 5, 6, 8, 11, 14, 15, and 17) would support the

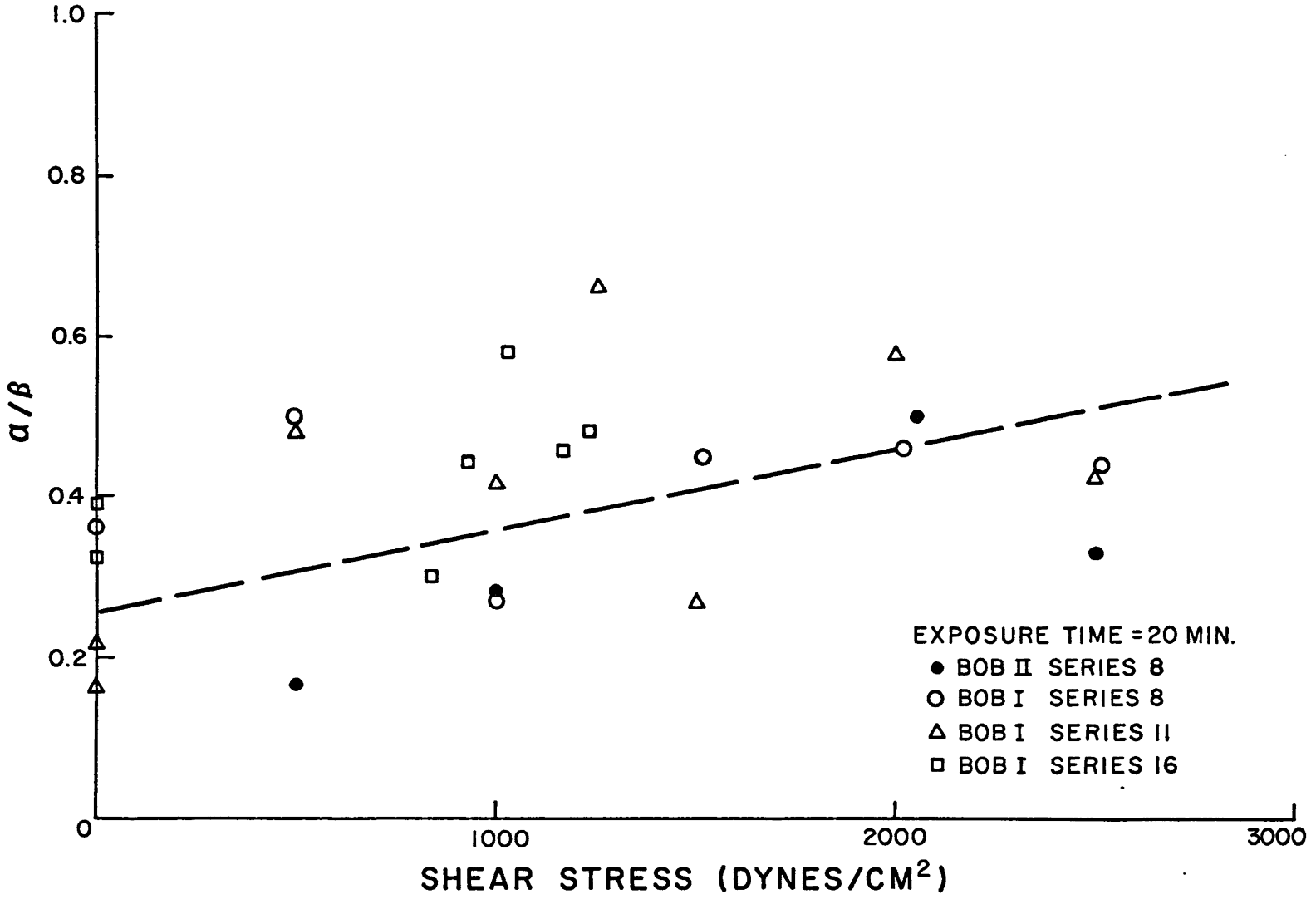


Figure 4A

Electrophoresis Gel Results,
Constant Exposure Time, Varying Shear Stress

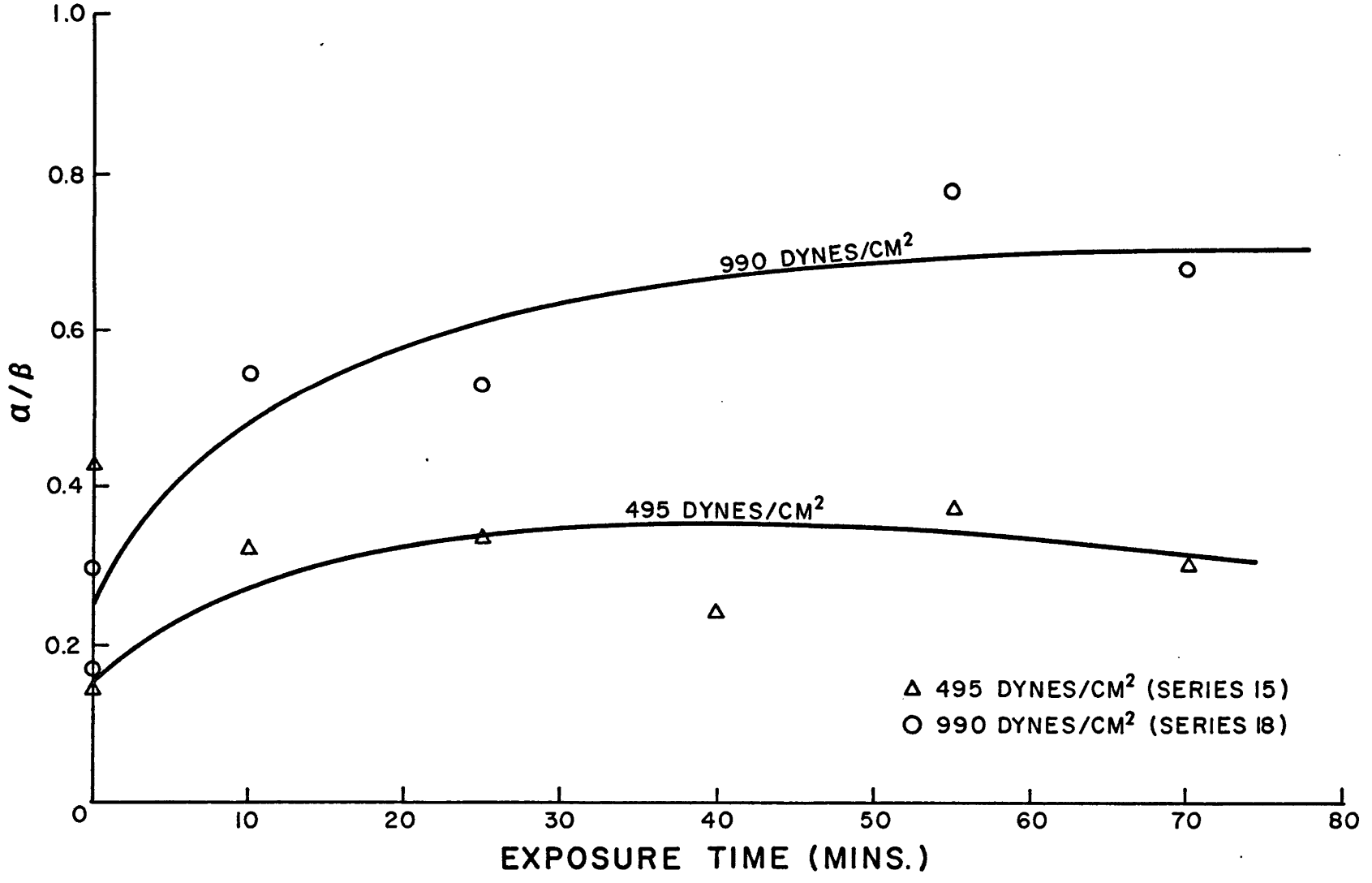


Figure 4B

Electrophoresis Gel Results,
Constant Shear Stress, Varying Exposure Times

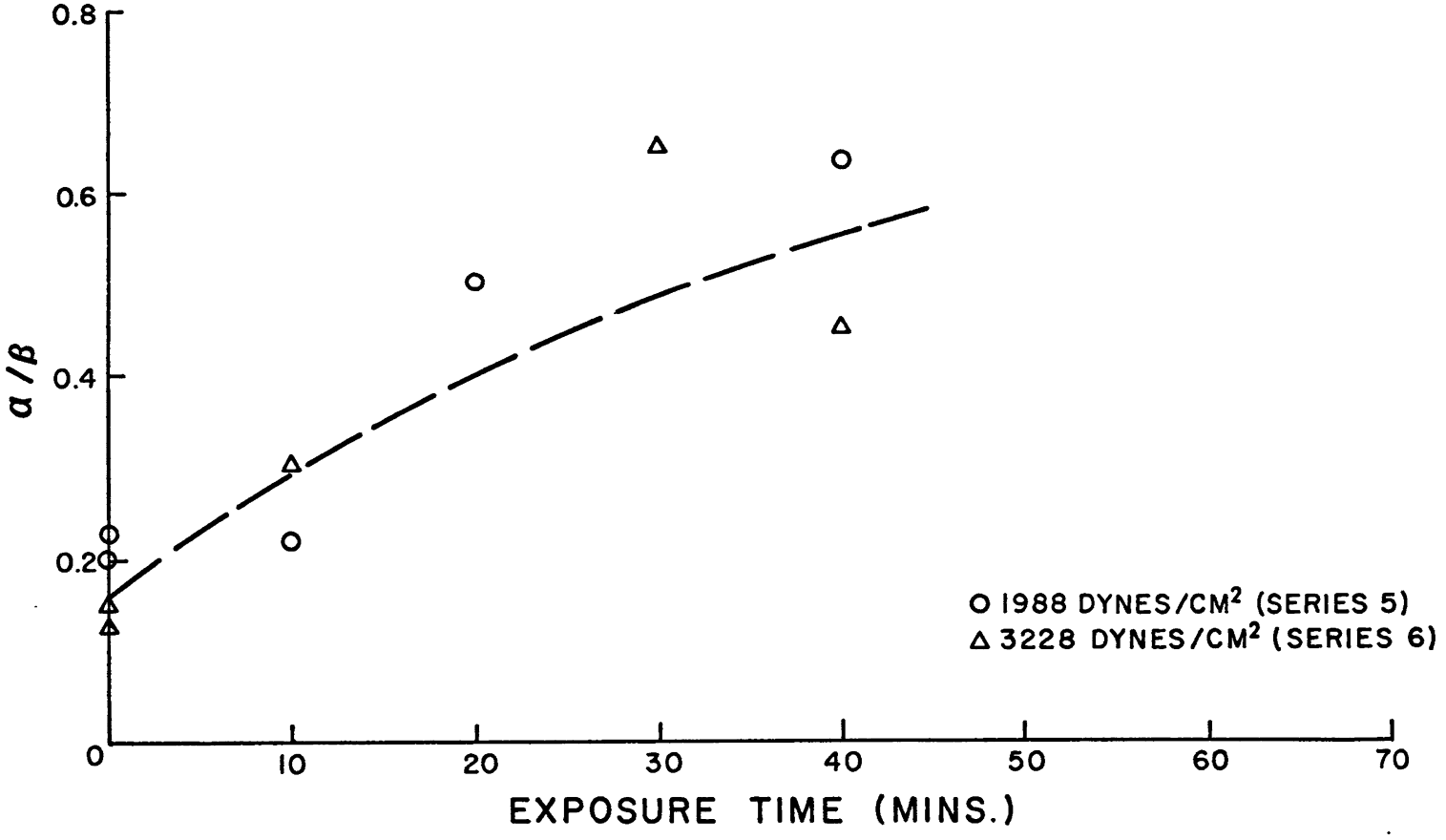


Figure 4C
Electrophoresis Gel Results,
Constant Shear Stress, Varying Exposure Time

postulate of an altered clotting mechanism. The gels were all prepared in the same manner, using calcium chloride to form the clot (see Appendix II, p. 4 for details). Analysis of the various band intensities in the gels was performed with a microdensitometer (Analco, Model D) with an integrating chart recorder (Hewlett-Packard, Model 7100-B) attached. The beta bands (showing beta polypeptide chain concentration) were used as the reference controls, and the ratios of the area under the alpha monomer peaks to the area under the beta peaks were compared for the various data points. Figures 4A - 4C were composites of the gel analyses for the constant time, varying shear stress studies and the constant shear stress, varying time studies respectively. It can be seen from these composites that, although the data is fairly scattered in Figure 4A, there is a general tendency of the alpha to beta ratios to increase with increasing shear stress and exposure time, indicating a decrease in the amount of alpha chain polymerization.

Error Sources

Although it was shown that the data was closely reproducible on any given day (see series 19, Table 6), as can be seen from the data (Appendix III), the day to day reproducibility was not particularly close. Both plasma and equipment problems are sources of this error.

Plasma Errors

The total fibrinogen content of the plasma pools varied about seventeen percent from day to day (from 273 MG% to 325 MG% according to the thrombin time peak values of controls), and the effects of this variable was not studied. Also, the effect of viscous heating during shear was assummed to be negligible, since the temperature was maintained between 18°C and 37°C for all runs.[5] Viscosity calculations for the plasma of different series showed that the viscosity varied somewhat from day to day (see Appendix I, p. 2 for details). Possibly all the bottles of plasma were not completely lyophilized, thus causing a problem in reproducing a plasma with consistent clotting characteristics. There were also the aforementioned problems in dissolving the lyophilized plasma. Still another source of error is the accuracy of the clotting tests, the best of which (thrombin time) was only accurate within five percent.

Equipment Errors

Before each series the viscometer was dismantled for cleaning. Once reassembled, the top and bottom gaps (see Figure 2) had to be reset. Due to the design of the viscometer, resetting these gaps consistently within ten percent of the design value was very difficult. Although this variable had little effect on the tested plasma samples (a 100% error in both gaps would result in less

TABLE 6

Series 19

T.T. Peak Analysis Bob I

Sample	Shear Rate (Sec^{-1})	Shear Stress ($dynes/cm^2$)	Exposure (min)	MG% Fib	% Clot	Comments
1	0	0	0	297	100	Control
2	43510-57430	793	40	248	835	
3	46800-50925	793	71	198	66.6	
4	47820-56670	793	55	186	62.6	
5	50850-56925	793	25	285	95.9	
6	47310-57180	793	25	285	95.9	
7	46550-55660	793	25	273	91.9	
8	0	0	0	297	100	Control
9	0	0	0	297	100	Control

than a two percent change in volume), it could have some effect on the average shear stress of the sample by increasing or decreasing the shear stress in the C-P and C-C regions, thus changing the torque on the bob, giving a slightly higher or lower torque reading (from which shear stress was calculated). Tests with standard viscosity oils showed this error to be a maximum of about one percent.

Since the cleaning procedure between runs of a given series did not require totally dismantling the viscometer, this problem did not exist for the various runs of a series. However, equipment vibration caused the cup assembly to drop, thus increasing the bottom gap and decreasing the top gap, and changing the volumes of both sections. The distance the cup would drop was dependent on the cup rpm. At low shear stresses ($<$ 500 dynes/cm^2) the drop was not noticeable, but at the higher shear stresses ($>$ 1000 dynes/cm^2) the drop could be as high as fifteen to twenty-five percent of the calculated top gap width (bob I). The exact gap change at a given cup rpm was not consistent, and would vary between runs and from series to series. With bob II this problem was not as evident, since the absolute change in gap widths was the same, but the percentage change was much smaller.

Constant Exposure Time, Varying Shear Stress Results

Although the data was not very reproducible for these series (4, 7, 8, 11, 12), each series was consistent

within itself. The plots of % clottability versus shear stress for these series show that the clottabilities decrease with shear stress from 0 to 1000 dynes/cm^2. At shear stresses above 1000 dynes/cm^2 the clottabilities increased with shear stress to about 1300 dynes/cm^2, at which point the points became fairly random. It was at shear stresses above 1300 dynes/cm^2 and below 2000 dynes/cm^2 that the system seemed to arbitrarily become turbulent during the shear run. At shear stresses above 2000 dynes/cm^2 the system appeared to be turbulent throughout the run. An apparent exception to this behavior is series 4. However, in this series no data was taken at shear stresses between 1000 and 2000 dynes/cm^2.

It should also be noted that for the constant time, varying shear series using bob II (with a smaller surface to volume ratio than bob I, see Table 1) a minimum in in clottability was also reached near a shear stress of 1000 dynes/cm^2, but that the magnitude of the % loss in clottability was much less than that incurred in the series using bob I (Figures 5, 6). This would suggest that the surface is a factor in decreasing the clottability. The minimum in these curves observed near 1000 dynes/cm^2 could be due to interaction of the molecules which would protect them from the forces acting on them to some extent. Possibly, conformational changes in the molecule hinder

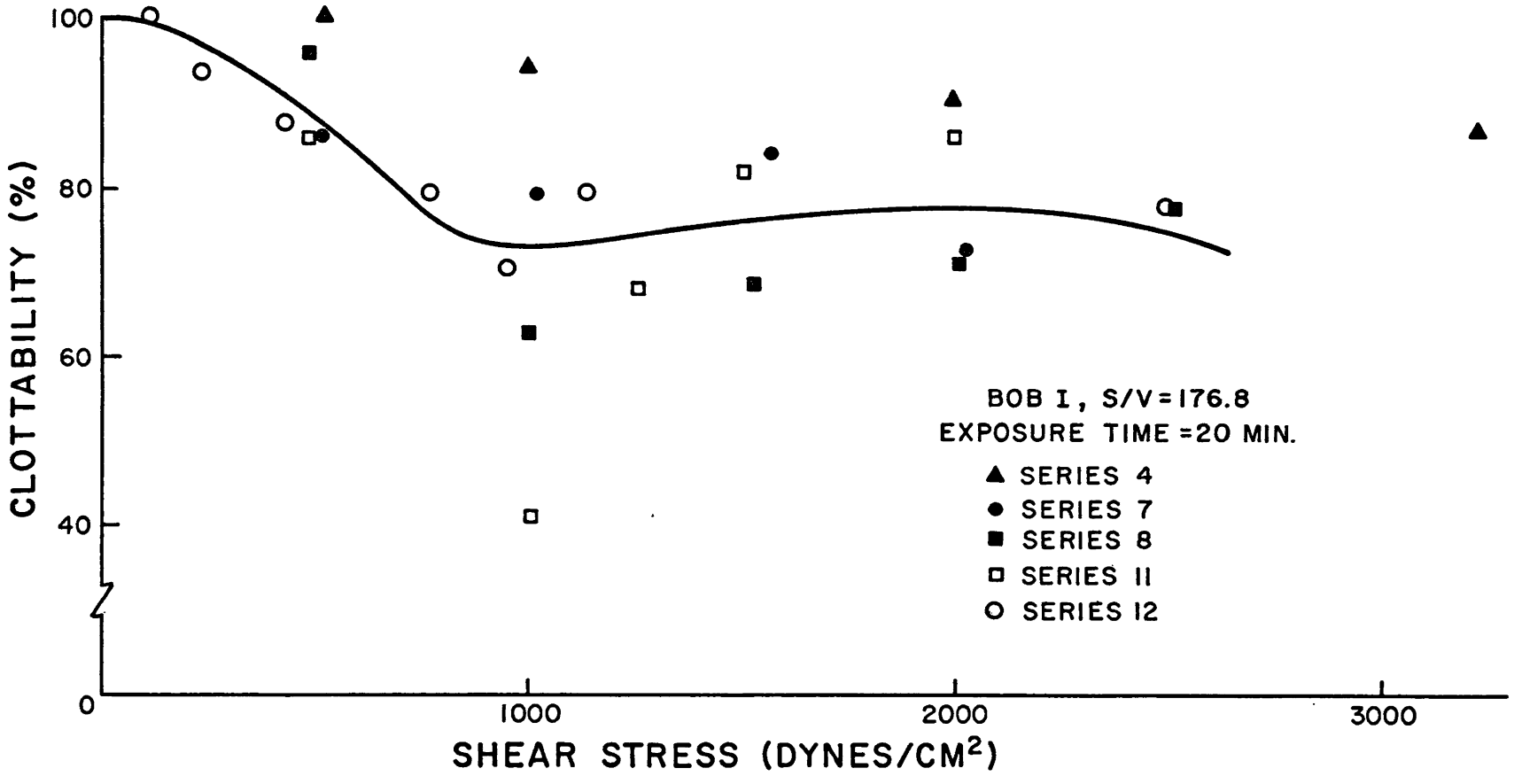


Figure 5

Constant Exposure Time,
Varying Shear Stress, Bob I

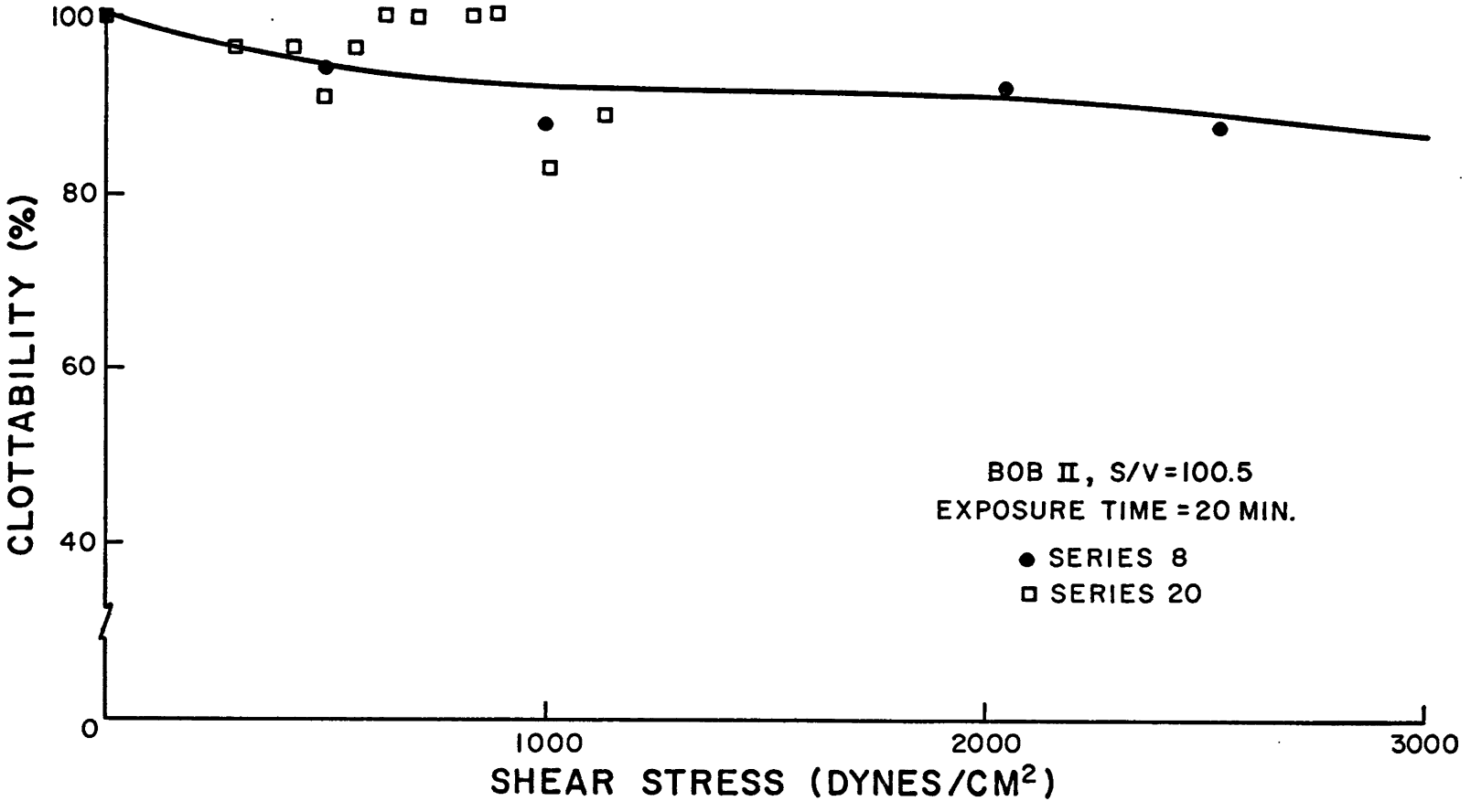


Figure 6

Constant Exposure Time,
Varying Shear Stress, Bob II

the availability of the bonding sites to cause the decrease in clottability, and at stresses higher than 1000 dynes/cm^2 the conformational changes are great enough to once again free some of the bonding sites, allowing increased clottability.

Constant Shear Stress, Varying Exposure Time Results

Analysis of the constant shear, varying time series (5, 6, 14, 16, 17, 18, 19, and 21) indicates that the clottability decreases when sheared for increasing time exposures. Also, the degree of clottability loss is highly dependent upon the shear stress used. The lowest shear stress used was 288 dynes/cm^2 (series 21), where no appreciable loss in clottability was observed for exposure times under 55 minutes, and the maximum loss at 70 minutes was only 7%. As the shear stress increases, the clottabilities decrease, and at the very high shear stresses (1988 dynes/cm^2 and 3228 dynes/cm^2) very noticable loss occurs (40% loss after 40 minutes). A composite plot of all series with % clottability versus exposure time (Figure 7) illustrates this idea, although the aforementioned problems in procedure and analysis prevent an accurate quantitative discussion of this tendency.

Theoretical Results

Using the method of Leventhal and Davison,[24] the stress exerted on a fibrinogen molecule by the viscometer

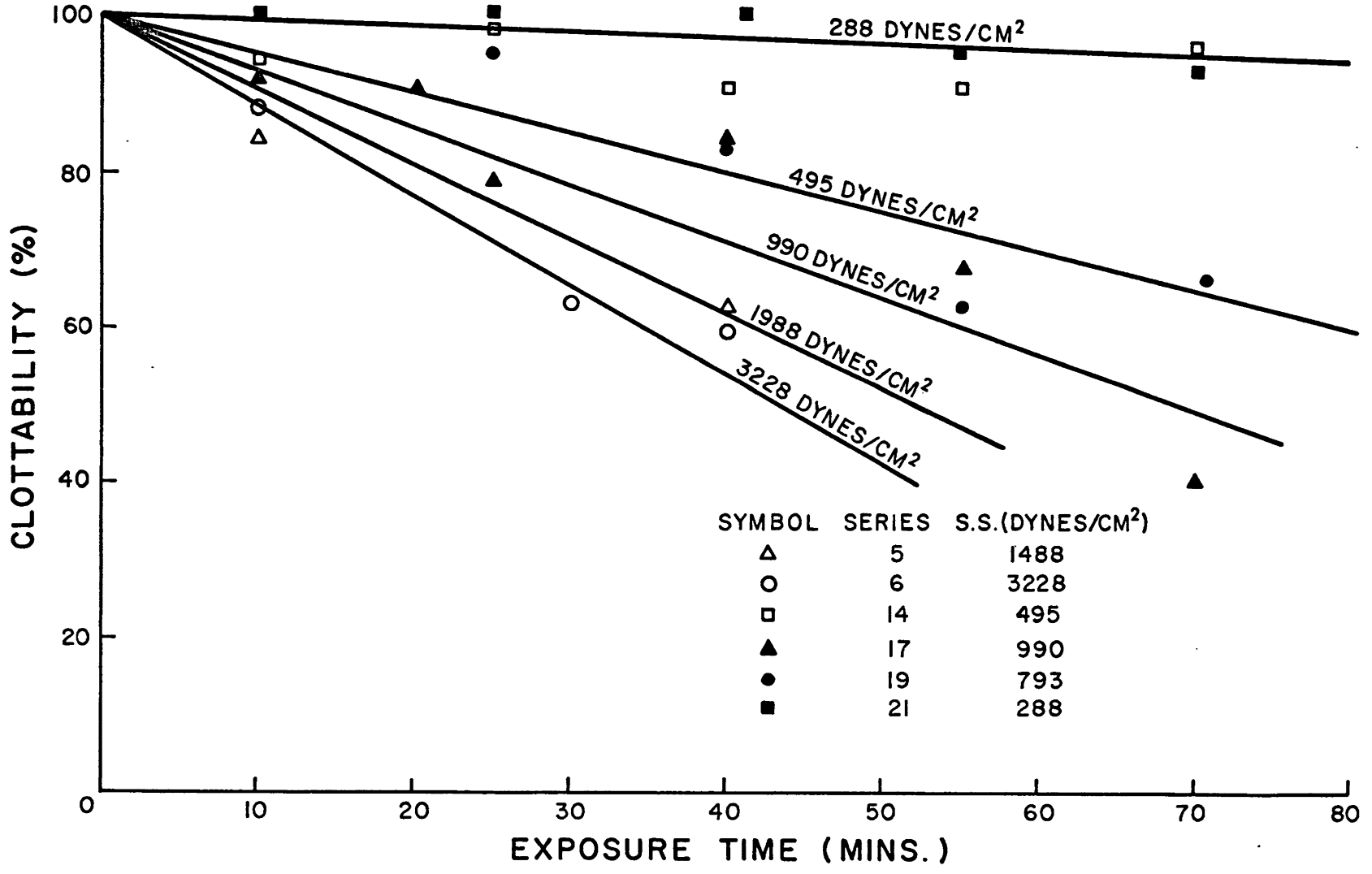


Figure 7

Constant Shear Stress,
Varying Exposure Time, Bob I

can be calculated. A fully extended molecule inclined at a 45^{o} angle to the streamlines in a uniform velocity gradient will experience a stretching force, τ , which will be maximized at the center of the molecule:

$$\tau_{max} = fGL^2/16$$

where G is the velocity gradient, L is the molecule length, and f is the frictional resistance per unit length of the molecule. f can be calculated using

$$f = 3\pi\eta/\log\left(\frac{L}{\delta}\right)$$

with η being the viscosity, L the molecule length, and δ the molecule diameter.

For the CSSV the velocity gradient can be assumed approximately uniform and equal to the shear rate. If the following values for these variables are used in the calculation of τ_{max},

$\eta = 1.4cp$

$G = 7.0 \times 10^4 \ sec^{-1}$

$L = 650 \ A^{o}$

$\delta = 65 \ A^{o}$ derived by hydrodynamic measurements[22]

A value of 2.5×10^{-8} dynes is the calculated maximum force on the molecule. Levinthal and Davison calculated the tensile strength for C-C and C-O bonds in DNA to be 8.1×10^{-4} and 8.9×10^{-4} dynes respectively. Assuming the tensile strength of the C-N and S-S bonds in fibrinogen to be two orders of magnitude smaller (10^{-6} dynes), still

the shear rates obtained in this study (< 200,000 sec^{-1}) would not be great enough to cause cleavage of the polypeptide chains (actual calculated bond energy values or C-N and S-S bonds are about 856 and 60% of the C-C bond energies respectively[16]). Thus, the loss in fibrinogen clottability with shear noted in this study and by Charm and Wong must be due to factors other than cleavage of the molecule. The electrophoresis gels made from samples obtained from the shear series would support this theory, since, had cleavage occurred, lower molecular weight bands than the beta band would have been present. Also, the total protein analysis (Biuret Method) of Series 11 illustrates that clottability loss can be achieved without destruction of the protein.

Comparison of Results with Those of Charm and Wong

Charm and Wong's method of measuring clottability was as follows:

> Clottability was measured by addition of thrombin (0.2 ml, 25 unit/ml) to plasma (0.5 ml) in a buffer (1.5 ml) containing 0.15M ammonium acetate, 0.1M 6-aminocaproic acid, and 17 mM Ca ion. The clot that formed after the sample was incubated for 3 hours at 37°C was washed and dissolved in 40 percent urea in 0.2N NaOH; its optical density at 280 nm ($O.D._{280}$) was read against a urea blank. The optical density of the sample was compared with that of plasma.[5a]

The method used in this study consisted of comparing the peak height of a sheared sample obtained when measuring thrombin time with a Bio-Data instrument

(product of Bio-Data, Hatboro, Pennsylvania) with the peak height of a control (unsheared) sample. This peak value is a measure of the maximum optical density of the clotted sample.

Charm and Wong determined that a mass average shear (80) of approximately 5×10^8 would produce a 50% loss in clottability. Assuming average shear rates of 1.02×10^5 sec^{-1} and 1.22×10^5 sec^{-1} for the constant shear runs at 1988 dynes/cm^2 and 3225 dynes/cm^2, and 52 and 43 minutes required times to produce a 50% decrease in these respective runs (see Figure 7) the mass average shear required to produce a 50% clottability loss would be approximately 3.2×10^8 using half-lives extropolated from Figure 7 for the runs at 793 dynes/cm^2 and 990 dynes/cm^2 and averaged shear rates of 5.2×10^4 sec^{-1} and 6.8×10^4 sec^{-1} respectively gave values of $\simeq 3 \times 10^8$ and $\simeq 2.8 \times 10^8$ for the required mass average shears of these runs. All these values are somewhat lower than that obtained by Charm and Wong (approximately 60% of their value).

Three obvious procedural variations in the experiments could be the source of this difference:

1) the difference in the magnitude of times under shear,
2) the differences in the magnitudes of the shear rates and

3) the differences in the analytical methods of measuring clottability.

Also, since the data using different surface to volume ratios indicates that there is a surface effect, this surface effect could provide still another reason for the difference in the two mass average shear values. The data showing less clottability loss with a lower surface to volume ratio would indicate that possibly a more similar value of the mass average shear to that of Charm and Wong could be obtained by simply changing this variable.

SUMMARY AND CONCLUSIONS

Plasma was sheared in a constant shear stress viscometer to determine the effects of shear on fibrinogen clottability. This work was divided into three areas: surface effects, shear effects, and time effects. Analyses were made using three different clottability measurements and electrophoresis gel analysis.

To determine if any fibrinogen was lost to the surface, Iodine^{125} labeled fibrinogen was sheared in the viscometer at various shear stresses, and samples were tested to see if any observable decrease in the amount of labeled fibrinogen occurred. Comparison of sheared samples with control samples indicated that no loss to the surface resulted from shearing.

Nine series of data were taken to determine the effect of different shear stresses on clottability for equal time exposure. Although the reproducibility was fairly poor, each series was self-consistent, since each showed a gradual decrease in clottability as shear was increased from 0 to 1000 dynes/cm^2. All series exhibited a minimum clottability at the points taken near 1000 dynes/cm^2, and the data showed rather erratic behavior at shears higher than 1000 dynes/cm^2, possibly due to the occurrence of turbulence during exposure. Decreasing the surface to

volume ratio of the sheared samples caused a lessening in the extent of clottability loss, indicating that surface effects on clottability loss were appreciable. It was assumed the minimum was caused by a maximum in the amount of hindrance to polymerizing site availability due to conformational changes, since no molecular destruction was observed.

Eight series were run where the same shear stress was used for the entire series, but the length of exposure time was varied. Analyses suggested that clottability loss was a function of both time and shear stress. Destruction increased with time, and with increased shear stress.

Calculations were made using the method of Levinthal and Davison to determine if sufficient stretching force on the molecule to cause scission of the polypeptide chains was present. Results showed that scission should not occur, and experimental results using gel electrophoresis and total protein analysis supported this theory. Gel analysis also showed that probably the alpha chain is the most affected by shear (see pages III-23 - III-26).

It can be concluded that fibrinogen clottability is affected by shear stress, but that loss is probably due to conformational changes rather than destruction of the molecule.

APPENDIX I

EQUIPMENT CALIBRATION DATA

APPENDIX I

I-A Bob Calculations

The micrometer used to measure the bob diameters gave a reading of 3.0002" on a standard of 3,0000", or an error of 6.66×10^{-3} percent. The values in Table 1 have been corrected for this error.

The volumes in the various sections or the viscometer were calculated on a geometrical basis, not allowing for any dead volume at the corners. Thus the actual volume in the sheared section is slightly higher than the volumes given in Table 1. MacCallum's method was used to calculate the surface to volume ratio and the shear rate (γ). For the surface to volume ratio, the equation used was:

$$\frac{A_t}{V_t} = \frac{2\left[Z(1+K) + \frac{(1+K)^2}{4K} + (1+\mathrm{CSC}^2\,\theta\,\mathrm{ave})^{\frac{1}{2}}\right]}{Ro(1-K^2)\left[Z + \frac{(1 + \mathrm{CSC}\,\theta\,\mathrm{ave})}{3K}\right]}$$

Where $Z = L/Ro$. For the shear rate,

$$\gamma_{avg} = \frac{\pi}{15}\,\frac{K}{1-K^2}\,(\mathrm{rpm})$$

$$\text{or}\quad \gamma_{avg} / \mathrm{rpm} = \frac{\pi}{15}\,\frac{K}{1-K^2}$$

Calculations of the theoretical "turbulent transition" points were also taken from MacCallum. Assuming an average plasma viscosity of 1.55 centipoise, and using

$$\frac{rpm_{trans}}{\nu} = \frac{1.91 \times 10^4}{Ro^2 (1-K)}$$

Where ν = kinematic viscosity. Since the density of platelet poor plasma is approximately equal to the density of water (1 gram/cc) ν can be replaced numerically with μ, and substitution of the k values for bob I and bob II yields rpm_{trans} points of

rpm_{trans}(bob I) = 6231 rpm

and rpm_{trans}(bob II) = 3545 rpm.

For bob I, evident turbulence was observed at 40-50% of this value, and for bob II at 60-65% of the calculated value. MacCallum and Lemuth attributed this "early" turbulence to eccentricity of the equipment and secondary flows in the conical sections.

I-B RPM Calibration

The cup rpm calibration was done by measuring the actual cup rpm with the Jacquets Indicator at various values of V_{rpm} and graphically determining the slope of V_{rpm} versus actual cup rpm. Approximately five readings were taken at each value of V_{rpm}, and the experimental values and the graphical representation can be found on pages 5 and 6 respectively.

I-C Torque Calibration

The torque calibration was made in two different ways. First the bob (III) was positioned on the viscometer and a string tied around the bob sufficiently tight to avoid slippage. The end of the string was put through a simple pulley (level with the string placement on bob) and weights of various sizes were hung from the string, noting the value of V_{torq} produced by each weight. Using the method of MacCallum, the shear stresses required to produce a torque equivalent to that produced by the weights were calculated for each bob. Next, Brookfield Standard Silicon Oil (5.0 cp at 25°C) was placed in the assembled viscometer, and V_{torq} was determined for given values of V_{rpm} and V_{temp}. The shear stresses produced by the fluid at the various values of V_{torq} were compared with those obtained from the "deadweight" measurments, and found to be within three percent. Therefore the "Dead Weight" measurements were used for the experimental shear stresses. The relation between actual shear stress and V_{torq} was determined using the method of Daniels, Williams, et. al. for a least squares fit.[10] Assuming the appropriate form is

$$\text{Actual shear stress} = M \times V_{torq} + B,$$

The values obtained for bob I and bob II were:

	bob I	bob II
M =	22.9706	23.0734
B =	-10.6622	-10.7075

The standard deviation for bob I was 6.658 and for bob II was 6.6836.

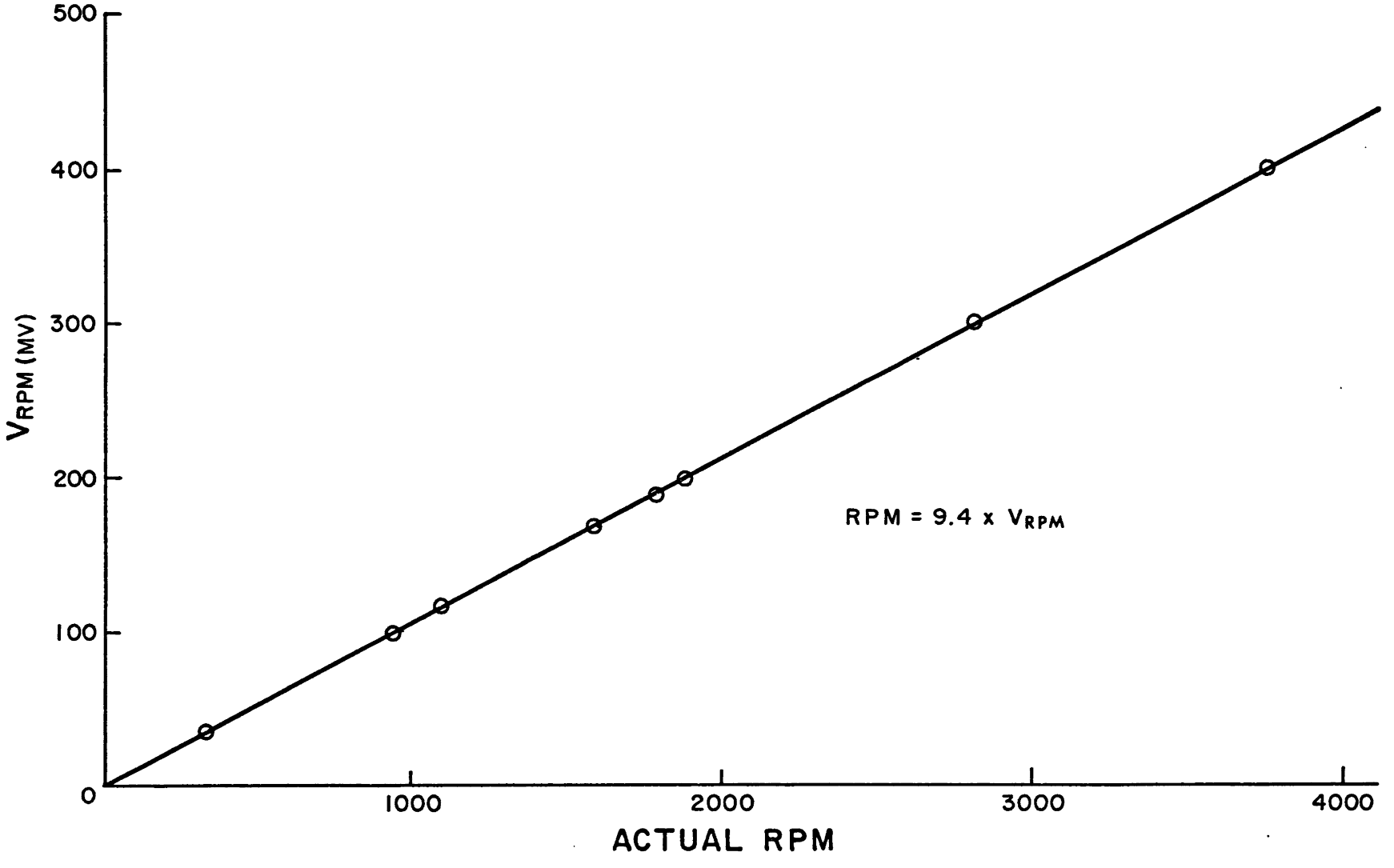
It should also be noted that the viscosity of a fluid being sheared can be determined by

$$\text{shear rate} \times \text{viscosity} = \text{shear stress, or}$$

$$\text{viscosity} = \frac{\text{shear stress}}{\text{shear rate}}$$ [25a]

V_{rpm} Calibration

V_{rpm}(mv)	Actual rpm
7	77
14	132
22	216
36	342
47	451
58	552
71	676
86	811
100	940
116	1098
129	1218
145	1360
170	1590
190	1790
200	1880
226	2130
250	2348
276	2600
300	2814
350	3290
400	3760
449	4225



RPM Calibration - Constant Shear Stress Viscometer

"Dead Weight" Calibration

V_{torq}(mv)	Mass(gm)	Torque (dyne-cm)	Shear Stress Bob I(d/cm^2)	Bob II(d/cm^2)
0	0	0	0	0
1	1.46	4948.56	6.84	6.87
2	6.46	21895.7	30.25	30.39
3	11.46	38842.8	53.66	53.91
4	16.46	55789.96	77.01	77.42
5	21.46	72737.1	100.49	100.94
6	26.46	89684.2	123.91	124.46
7	31.46	106631.4	147.32	147.98
8	36.46	123578.5	170.74	171.50
9	41.46	140525	194.15	195.02
10	46.46	157472.8	217.56	218.54
11	51.46	174419.8	240.98	242.06
12	56.46	191367.0	264.30	265.58
13	61.46	208314.2	287.8	289.1
15	71.46	242208.4	334.64	336.13
17	81.46	276102.7	381.46	383.17
19	91.46	309996.9	428.29	430.21
23	111.46	377785.5	521.95	524.29
31	151.46	513362.5	709.3	712.44
37	181.46	615045.3	849.75	853.55
41	201.46	682833.9	943.40	947.63

"Dead Weight" Calibration (Continued)

V_{torq}(mv)	Mass(gm)	Torque (dyne-cm)	Shear Stress Bob I(d/cm^2)	Bob II(d/cm^2)
43+	211.46	716728.1	990.23	994.67
52	255.5	865998.5	1196.46	1201.82
95	464.5	1578388.6	2175.18	2184.92
186	913.5	3096241.1	4277.77	4296.93
283	1378.0	4670629.7	6452.94	6481.85

Experimental Calibration - Bob I

Brookfield Standard Silicon Oil

V_{rpm}	V_{temp}	μ(cp)	V_{torq}(mv)	Shear Stress (dynes/cm^2)
0	1153	5.30	000	0
20	1167	5.22	12	264.12
30	1172	5.20	18	394.66
40	1200	5.17	23	523.17
50	1270	5.03	28	636.26
60	1330	4.70	31	713.42
80	1370	4.65	41	941.11
100	1416	4.60	52	1168.55
150	1530	4.51	75	1711.45
200	1650	4.38	97	2216.16
250	1845	4.05	114	2561.48
300	1900	3.90	134	2959.94
200	1725	4.20	95	2175.08
100	1550	4.50	52	1138.54
50	1450	4.6	28	588.19
0			0	0
100	1340	4.70	52.5	1189.03

Experimental Calibration - Bob II

Brookfield Standard Silicon Oil

V_{rpm}	V_{temp}	μ(cp)	V_{torq}	Shear Stress (dynes/cm^2)
0	849	5.94	0	0
12	850	5.94	41	102.5
20	850	5.94	8	170.8
30	853	5.94	11	255.8
40	859	5.93	14	341.0
52	862	5.93	18	443.3
62	875	5.93	22	528.6
82	915	5.92	28	698
102	949	5.92	34	868
120	968	5.91	39	1020
136	1003	5.905	44	1155
150	1014	5.905	52	1277
170	1029	5.87	57	1435
190	1060	5.85	62	1598
210	1078	5.80	71	1751
230	1100	5.75	79	1918
250	1120	5.6	86	2013
252	1122	5.6	87	2029
270	1136	5.5	92	2135

APPENDIX II

ANALYSIS METHODS

APPENDIX II

Four methods were used to obtain the clottabilities of the sheared samples. For the first three series, analyses were made using the tyrosine test of Ratnoff and Menzie, but no consistent results could be discerned. A pool of plasma was made and divided into three groups; and eight samples taken from each group. The samples from one group were tested immediately. Of the other two groups, one group was frozen for two days, one group was refrigerated for two days. The fresh samples indicated an inherent 10% error, and both freezing and refrigeration caused a change in the measured clottabilities. The Biuret technique of fibrinogen determination is also a total protein determination, and showed no observable changes in clottability.

The method used for the majority or the clottability determinations was analysis of peak heights obtained with thrombin time determinations with the bio-data instrument. The peak heights were proportional to the total clottability by the ratio

$$\frac{25}{310} = \frac{\text{T.T. Peak Height}}{\text{mg\% fibrinogen}} = \text{constant}$$

On some of the series electrophoresis gel analysis was also used. Gel analysis was done with a micro-densitometer with the output attached to an integrating chart recorder.

BII-A Refrigeration Effects

One half unit of blood was taken from a donor and placed in a Pliapak bag containing 3.2% sodium citrate solution (9/1 ratio of blood to sodium citrate solution). After centrifuging to obtain platelet poor plasma, the plasma was split into 24 samples, and these samples were divided into three groups. Clottabilities for the samples of one group were determined immediately, while one of the other groups was placed in a refrigerator at $5^{o}C$ and the last group placed in a freezer at $18^{o}C$. Both of the latter groups were tested in the same fashion after two days. The following data gives the results of these tests.

Fresh plasma

Sample	MG% Fibrinogen (two tests)	
1	133	164
2	147	145
3	138	152
4	145	159
5	147	145

Sample	MG% Fibrinogen (two tests)	
Fresh plasma cont.		
6	159	143
7	161	145
8	138	145
Refrigerated plasma		
1	185	
2	173	
3	183	
4	187	
5	164	
6	168	
7	192	
8	183	
Frozen plasma		
1	161	
2	187	
3	150	
4	176	
5	164	
6	164	
7	145	
8	173	

II-B Electrophoresis Gel Preparation

The gels for all series where gels were prepared were made in the same manner. The following is the procedure used in preparing the gels for series 6.

1. Five samples of normal plasma, were clotted as follows:
    1. 0.128 ml Sample 1 + .064 ml $CaCl_2$(.01M) + .064 ml Thrombin (20u/m)
    2. 0.128 ml Sample 2 + .064 ml $CaCl_2$(.01M) + .064 ml Thrombin (20u/m)
    3. 0.128 ml Sample 3 + .064 ml $CaCl_2$(.01M) + .064 ml Thrombin (20 u/m)
    4. 0.128 ml Sample 4 + .064 ml $CaCl_2$(.01M) + .064 ml Thrombin (20 u/m)
    5. 0.128 ml Sample 5 + .064 ml $CaCl_2$(.01M) + .064 ml Thrombin (20 u/m)
2. Gels were incubated @ 37°C for 24 hrs. and then washed twice in NaCl (Washed clots were frozen @ -20°C in NaCl until ready to incubate)
3. Each clot was blotted on filter paper and then incubated in IB @ 37°C overnight.
4. Electrophoreas on SDS gels. (15-20 ul/gel)

Thrombin 5000 µ reconstituted in 1 ml distilled H_2O; then diluted to 20 u/ml in barbital buffer, ph 7.5

.01M $CaCl_2$	.0147 g in 10 ml barbital buffer, ph 7.5
	(use $CaCl_2 \cdot 2H_2O$ - ph remained 7.5)
.02M EDTA	.0744 g in 10 ml barbital buffer. (ph 7.5)
	Adjust ph of final soln. to ph 7.5 to HCl

Biuret Analysis

PLASMA FIBRINOGEN CONCENTRATION

Collect plasma from whole blood anticoagulated in citrate or oxalate. In a test tube add 2.0 ml plasma, 2 ml 0.025 M $CaCl_2$, 5 ml saline and 0.2 ml rabbit brain thromboplastin. Allow to clot completely at room temperature for 30 min. Now express plasma from the clot, and remove it. Wash the fibrin clot twice with 5 ml portions of saline. Add 5 ml biuret reagent to the clot and incubate at 37°C for 30 min. Centrifuge for 5 min at 2500 rpm to remove any undissolved debris and read optical density at 540 μ (54 filter in Klett colorimeter). Use the biuret reagent as the blank.

The standard solution may be any protein solution of known concentration. For example, Versatol with a total protein of 7300 mg/100 ml. Dilute the reconstituted Versatol 1 ml with 2 ml saline. This gives a solution of protein concentration about 2433 mg/100 ml. Add 0.2 ml (4.9 mg protein) to 5 ml biuret reagent and allow to incubate for 30 min with the unknown. The calculations then are:

$$\frac{\text{optical density of unknown}}{\text{optical density of standard}} \times 4.9 \times 1/2 \times 100 =$$

plasma fibrinogen in mg/100 ml

Reagents:

1. Biuret reagent: On the day of the test add 5 ml 1% $CuSO_4$ and 25 ml 12.5% NaOH. The solution is stable for several hours.

Comments: If the unknown does not clear with centrifugation, add a few drops of ether, agitate for several minutes and again centrifuge. The ether layer then can be aspirated or a sample pipetted from beneath the layer and placed in the cuvet.

II-D Tyrosine Test for Fibrinogen Concentration

Determination of Ratnoff and Menzie [28]

APPARATUS AND REAGENTS:

Pipets, 1.0 ml. graduated in 0.01 ml. CHEMICALLY CLEAN
Pipets, 1.0 ml. volumetric, CHEMICALLY CLEAN
Pipets, 5.0 ml. graduated in 0.1 ml., CHEMICALLY CLEAN
Pipets, 10.0 ml. graduated in 0.1 ml., CHEMICALLY CLEAN
Ignition tubes, heavy wall, Pyrex, 100 x 14 mm. diameter, CHEMICALLY CLEAN
Centrifuge tubes, round bottom, constricted neck tube, 40 ml., heavy duty, Pyrex, CHEMICALLY CLEAN
Test tubes, 150 x 18 mm. diameter, CHEMICALLY CLEAN
Beckman cuvettes, 75 x 12 mm. diameter, CHEMICALLY CLEAN
Capillary pipets, CHEMICALLY CLEAN, with rubber bulb
Glass stirring rods, CHEMICALLY CLEAN
Crushed glass 50-100 mesh, CHEMICALLY CLEAN
Rubber bulbs, 5 ml. capacity
Corks, regular length, number 6
Water bath at 100°C
Stopwatch
Centrifuge
Beckman spectrophotometer

0.85 per cent sodium chloride solution (physiological saline)
10 per cent (W/V) sodium hydroxide solution
20 per cent (W/V) sodium carbonate solution
Folin and Ciocalteau phenol reagent (Hartmann-Leddon). Store in the refrigerator
Tyrosine standard solution containing 200 mgm. tyrosine per 1000 ml. 0.1 normal hydrochloric acid solution (HartmannOLeddon). Store in the refrigerator.
Thrombin solution (Parke Davis) containing 1000 NIH units per milliliter. Store in the freezer. Do not use thrombin solution which is more than two weeks old.

THE BLOOD SPECIMEN: Plasma fibrinogen are performed on venous blood to which a dry anticoagulant has been added.

NOTE: Plasma fibrinogens are always determined in duplicate.

PLASMA FIBRINOGEN DETERMINATIONS

PREPARATION OF THE FIBRIN CLOT:

(1) Transfer the blood specimen to an ignition tube, and centrifuge the blood at about 2000 revolutions per minute for 10-15 minutes to separate the plasma from the cells.
(2) With a capillary pipet, transfer the plasma to a test tube.
(3) Place a strip of adhesive tape on each of three 40 ml. round-bottom Pyrex centrifuge tubes. On each of two of the strips of tape write the name of the patient and the date; on the third strip write "fibrin blank"* and the date.
(4) Measure approximately 1 ml. of crushed glass (contained in a graduated centrifuge tube) into each of the centrifuge tubes.
(5) With a 10.0 ml. pipet, add 10.0 ml. of 0.85% sodium chloride solution to each centrifuge tube; with a 1.0 ml. graduated pipet, add 0.1 ml. of thrombin solution, and gently rotate each tube to mix the contents.
(6) With a 1.0 ml. graduated pipet, quickly add 1.0 ml. of plasma to one of the tubes labeled with the patient's name (will be referred to as "unknown"). Immediately agitate the tube with an oscillatory motion so that, as clotting occurs, the fibrin strands are trapped on the particles of glass. Agitate the tube in this manner for THREE MINUTES. If necessary, 0.5 ml. of plasma may be used for the determination.
(7) Record the amount of plasma used on the label.
(8) Using another 1.0 ml. graduated pipet, repeat steps (6) and (7) with the other "unknown" tube.
(9) Allow the tubes to stand for ten minutes. Observe the "unknown" several times during this period, and if further clotting occurs, repeat agitation for at least one minute.
(10) Centrifuge the tubes, including the tube containing the "fibrin blank", at about 2000 revolutions per minute for five minutes.
(11) With a 10.0 ml. pipet attached to a large rubber bulb, carefully remove the supernatant fluid from each "unknown".
(12) Test the supernatant solution removed from each "unknown" for any fibrinogen not clotted by adding several milliliters of the supernatant solution to a test tube containing about 0.2 ml. of thrombin solution. If coagulation occurs, discard the "unknowns", and start again at step (1).
(13) With another 10.0 ml. pipet, remove and discard the supernatant solution from the "fibrin blank".

* prepare only one fibrin blank for a series of determinations set up at the same time.

WASHING THE FIBRIN CLOT:

(1) With a 10.0 ml. pipet, add 10.0 ml. of 0.85% sodium chloride solution to each centrifuge tube, rinsing down the wall of the tube with the solution as it is added.
(2) In the "unknown" samples, carefully press the fibrin clot against the wall of the tube with a stirring rod. The clotted mass should be broken up so that any serum trapped within the clot will be released into the sodium chloride solution. However, rigorous attempts to separate the fibrin strands from the glass particles should not be undertaken.
(3) Centrifuge the tubes at 2000 revolutions per minute for three-five minutes.
(4) With a 10.0 ml. pipet attached to a large rubber bulb, carefully remove and discard the supernatant fluid from each "unknown", with another 10.0 ml. pipet, remove and discard the supernatant solution from the "fibrin blank".
(5) Repeat steps (1), (3) and (4).
(6) At this point, the tubes may be stoppered with corks stored in the refrigerator. The procedure can be continued when convenient.

DIGESTION OF THE FIBRIN CLOT:

(1) Do not run more than four determinations (eight "unknown" tubes) at one time. Use only one fibrin blank for each set of unknown determined at the same time.
(2) With a 1.0 ml. graduated pipet, add 1.0 ml. of 10% (W/V) sodium hydroxide solution to each centrifuge tube.
(3) Heat the tubes in a boiling water bath for TEN MINUTES. Loosely cover the tubes with a piece of filter paper to minimize evaporation.
(4) Remove the tubes from the bath, and allow them to cool completely.

ADDITION OF PHENOL REAGENT TO THE FIBRIN SOLUTIONS AND PREPARATION OF TYROSINE STANDARDS:

(1) Turn on the Beckman spectrophotometer.
(2) Label each of two clean 40 ml. round bottom centrifuge tubes "tyrosine standard". Label another 40 ml. centrifuge tube "tyrosine blank".
(3) Add the quantities indicated of the following reagents to each tube. Add the reagents in the order listed; after the addition of each reagent, gently rotate each tube to mix the contents.

REAGENTS	TUBES			
	UNKNOWN	FIBRIN BLANK	TYROSINE STANDARD	TYROSINE BLANK
(a) Tyrosine standard solution, ml. (Use 1.0 ml. volumetric pipet measure)	-	-	1.0	-
(b) 10% (W/V) sodium hydroxide solution, ml. (use 1.0 ml. graduated pipet to measure)	already present	already present	1.0	1.0
(c) Distilled water, ml. (use 10.0 ml. graduated pipet to measure)	7.0	7.0	6.0	7.0
(d) 20% (W/V) sodium carbonate solution, ml. (use 5.0 ml. graduated pipet to measure	3.0	3.0	3.0	3.0
(e) Folin-Ciocalteau phenol reagent ml. (use 1.0 ml. graduated pipet to measure)	1.0	1.0	1.0	1.0

(4) Centrifuge the tubes containing glass particles at 2000 revolutions per minute for about one minute.
(5) Allow the tubes to stand undisturbed for TEN MINUTES.
(6) With a 10.0 ml. graduated pipet, add 4.0 ml. of distilled water to each of a series of large test tubes labeled to correspond to the centrifuge tubes.
(7) With a 5.0 ml. graduated pipet, transfer 4.0 ml. of the solution in each centrifuge tube to the correspondingly labeled test tube. Use a separate 5.0 ml. pipet for each transfer. If the centrifuge tube contains packed crushed glass, be careful not to insert the pipet all the way to the bottom of the tube thereby disturbing the packed glass. Thoroughly mix the contents of the test tube by alternately withdrawing a portion of the solution into the pipet and expelling the solution into the tube.
(8) Allow the diluted solutions (1:2 dilutions) to stand undisturbed for an additional FIFTEEN MINUTES.

READING THE OPTICAL DENSITY OF THE FIBRIN AND TYROSINE SOLUTIONS:
(1) Transfer approximately 4 ml. of each diluted solution to a series of matched Beckman cuvettes.
(2) Set the wave length at 750 mu.
(3) Read the optical density of the tyrosine standard solutions and the fibrin blank solution against the tyrosine blank solution.
(4) Read the optical density of the fibrin solutions ("unknowns") against the fibrin blank solution.

(5) If the optical density reading of any of the "unknown" solutions is greater than 1.500, prepare a more dilute solution; e.g., a 1:4 dilution. This is accomplished by adding 2.0 ml. of undiluted solution from the "unknowns" and "fibrin blank", to 6.0 ml. of distilled water following the same general procedure outlined in steps (6) and (7) in the preceding section and then repeating steps (3) and (4) of this section. If it is necessary to make this dilution, multiply the final answer by two.

CALCULATION OF THE PLASMA FIBRINOGEN:

(1) Calculate the fibrinogen using the following formula:

y	optical density of the fibrin solution (unknown)
x	optical density of the tyrosine standard solution (use the mean of the duplicate solutions)
0.2	amount of tyrosine in the standard (0.2 mgm./ml.)
11.7	constant $\frac{\text{weight of fibrin}}{\text{"tyrosine-like" activity}}$
100	factor to convert the amount of fibrinogen in $\frac{1.0 \text{ ml. of plasma}}{\text{of plasma}}$ to the amount of fibrinogen in 100 ml.

$\frac{y}{x}$(0.2 mgm./ml.) (11.7) (100) = mgm.% fibrinogen

The product of (0.2) (11.7) (100) = 234

Therefore, the simplified formula is:

$\frac{y}{x}$ (234) = mgm.% fibrinogen

(2) The final reported result should be the mean of the duplicate determinations.

II-E

METHOD OF DETERMINING CLOTTABILITY OF PURFIED FIBRINOGEN

1. Fibrinogen Concentration in the sample is determined by the tyrosine method of Ratnoff and Menzie. Best concentration for clottability test is 100-400 mg%.

2. Set up in duplicate:
   (a) 0.5 ml fibrinogen solution + 0.1 ml thrombin (20 u/ml)
   (b) 0.5 ml fibrinogen solution + 0.1 ml saline (0.9%)

3. Rim (but do not remove) Clot after 10 minutes.

4. Allow clot to form at room temperature for 3-4 hours.

5. Add 2.5 ml 0.9% saline to each tube. Trap clot on wire loop, dip several times in the saline and remove. Clarify solution by centrifugation if needed.

6. Read O. D. of samples at 280 mu.

7. $$\% \text{ clottability} = 100 - \left[\frac{\text{O.D. clotted fibrinogen}}{\text{O.D. non-clotted fibrinogen}} \times 100\right]$$

II-F

Cr^{51} Analysis

In order to determine the effectiveness of the cleaning procedure, two pools of plasma cone containing Cr^{51} were made and alternately placed in viscometer. Both clean and isotope samples were counted to determine if appreciable residue was not removed. Points and counts are given for each in order performed, with cleaning between each point.

A-1: Sample of Cr^{51} pool
B-1: Sample of "clean" pool

A-2: Cr^{51} sample placed in CSSV, turned by hand
B-2: "clean" sample placed in CSSV, turned by hand

A-3: Cr^{51} sanoke ub CSSV, sheared at 1370 dynes/cm^2 (15 mins)
B-3: "clean" sample in CSSV, sheared at 1347 dynes/cm^2 (15 mins)

A-4: Cr^{51} sample in CSSV, sheared at 2773 dynes/cm^2 (15 mins)
B-4: "clean" sample in CSSV, sheared at 2773 dynes/cm^2 (15 mins)

Analysis:

Sample	Count	Sample	Count
A-1	304,001	B-1	none
A-2	292,739	B-2	1368
A-3	301,110	B-3	3217
A-4	292,284	B-4	4779

APPENDIX III

REDUCED SHEAR DATA AND

ELECTROPHORESIS GEL RESULTS

Series 1

Tyrosine Test Bob II 10 min/sample

Sample	Shear Rate (sec^{-1})	Shear Stress ($dynes/cm^2$)	O.D. (1st Test)	MG% Fibrinogen	O.D. (2nd Test)	MG% Fibrinogen
1	0	0	1.035	374	.603	210
2	200	23	.826	296	.530	185
3	28760	510	.585	209	.588	205
4	45575	2106-2035	1.013	362	.548	191
5	70754	3210-3050	.958	343	.589	205

Series 2

Tyrosine Test Bob II, 10 mins/sample

Sample	Shear Rate (sec^{-1})	Shear Stress ($dynes/cm^2$)	O.D.	Mg% Fibrinogen
1	0	0	.419	147
2	24447	519	.465	164
3	39690	1000	.445	157
4	50333	1760	.503	176
5	57811	2265	.508	178
6	71900	3256	.450	157
7	64715	2770	.440	154
8	89156	4475	.500	176
9	0	0	.458	161

Series 3

Tyrosine Test Bob I

Sample	Shear Rate (sec^{-1})	Shear Stress ($dynes/cm^2$)	O.D.	MG% Fib	Comments
1	126500	3645	.563	171	20 mins shear
4	126500	3645	.598	180	20 mins shear
5	126500	3645	.586	178	20 mins shear
2	0	0	.526	159	control
8	0	0	.656	199	control
14	0	0	.593	180	control
3	127765	3670	.694	211	60 mins shear
9	127765	3670	.666	201	60 mins shear
11	127765	3670	.600	180	60 mins shear
6	0	0	.668	201	place in CSSV, not sheared (15 mins)
7	0	0	.634	192	place in CSSV, not sheared (15 mins)

Series 3 Continued

Sample	Shear Rate (sec^{-1})	Shear Stress ($dynes/cm^2$)	O.D.	MG% Fib.	Comments
15	0	0	.329	98	place in CSSV, not sheared (15 mins)
10	0	0	.613	185	control
12	0	0	.666	201	control
13	0	0	.602	183	control

Series 4

T.T. Peak Analysis 20 mins/sample Bob I

Sample	Shear Rate (sec^{-1})	Shear Stress ($dynes/cm^2$)	Max Temp (^oC)	MG%T.T.	%Clot	MG% Biuret	Comments
1	0	0	21.0	255	100	300	control
2	0	0	21.0	255	100	265	
3	31623 - 33647	520	22.7	255	100	265	
4	67041 - 73619	1000	26.8	240	94.1	265	
5	123963	3220	31.4	220	86.3	300	
6	107013 - 99676	1988	26.6	230	90.2	232	
7	0	0	21.0	265	104	325	control

Series 5

T.T. Peak Analysis Bob I

Sample	Shear Rate (sec^{-1})	Shear Stress ($dynes/cm^2$)	Max Temp (^oC)	MG%T.T.	%Clot	MG% Biuret	Comments
1	0	0	22.0	255	100	265	control
2	187209	5217	51	0	0	125	20 mins
3	104483 - 98917	1988	33.9	163	62.7	275	40 mins
4	102459 - 100182	1988	27.0	218	83.8	250	10 mins
5	107013 - 102459	1988	27.0	236	90.8	275	20 mins
6	0	0	22.0	260	100	250	control

Series 6

T.T. Peak Analysis Bob I

Sample	Shear Rate (sec^{-1})	Shear Stress ($dynes/cm^2$)	Max Temp (^oC)	MG% Fib	%Clot	MG% Biuret	Comments
1	0	0	22.0	290	100	233	control
2	121686 - 123457	3228	34.1	255	87.9	275	10 mins
3	123710	3250	32.0	173	59.7	275	40 mins
4	122698 - 122192	3228	31.6	182	62.8	233	30 mins
5	0	0	22.0	290	100	265	control

Series 7

T.T. Peak Analysis, 20 mins/sample Bob I

Sample	Shear Rate (sec^{-1})	Shear Stress ($dynes/cm^2$)	Max Temp (°C)	MG% Fib	%Clot	Comments
1	0	0	22.0	273	100	control
2	30611 - 32129	518	24.5	235	86.1	
3	67297 - 77160	1023	25.5	217	79.5	
4	108783 - 101194	2034	26.0	198	72.5	
5	94616 - 98664	1574	31.6	229	83.9	
6	0	0	22.0	273	100	control

Series 8

T.T. Peak Analysis 20 mins/sample Bob I & Bob II

Sample	Shear Rate (sec^{-1})	Shear Stress ($dynes/cm^2$)	Max Temp (^oC)	MG% Fib	%Clot	Comments
1	0	0	22.0	297	100	Control
2	28334 - 29852	495	23.7	285	95.9	
3	31117 - 37695	1000	26.8	186	62.6	
4	94869 - 97400	89810 - 1529	31.8	204	68.7	
5	104989 - 101194	2011	34.6	211	71.0	
6	111313 - 106001	104230 - 2516	36.4	229	77.1	
7 (Bob II)	24441 - 30911	497	25.2	279	93.9	
8 (Bob II)	40257	1005	28.9	260	87.5	
9 (Bob II)	55928 - 59666	2043	34.7	273	91.9	
10 (Bob II)	62829 - 65704	0	36.4	260	87.5	
11	0	0	22	297	100	control

Series 9

I^{125} Labeled Fibrinogen 20 mins/sample 2 tests/sample Bob I

Sample	Shear Stress (dynes/cm^2)	Vol_1(ml)	$Count_1$	Vol_2(ml)	$Count_2$	Comments
1	0	0.9	338297	0.9	348695	control
2	0	0.9	327005	0.9	332560	control
3	495	0.9	320004	0.9	333930	
4	1000	0.9	324795	0.9	331295	
5	1508	0.9	330635	0.9	332305	
6	2037	0.9	346835	0.9	356709	
7	2520	0.9	347098	0.9	356709	

Series 10

I^{125} Labeled Fibrinogen 20 mins/sample Bob I

Sample	Shear Stress	Vol_1	$Count_1$	Vol_2	$Count_2$	Comments
1	0	0.9	331597	0.3	116627	control
2	472	0.9	330439	0.9	349340	
3	1023	0.9	315903	0.9	333876	
4	1505	0.9	344506	0.9	323812	
5	2034	0.9	335857	0.9	344843	
6	2492	0.9	350648	0.9	352349	
7	0	0.9	354583	0.9	326254	control
8	0	0.9	000315	0.9	000881	blank

Both Series 9 and Series 10 samples came from the same plasma pool.

The points 315903 (series 10, no 4) and 356709 (series 9, no 7) can be proven statistically acceptable using the method of Johnson and Leone

For 315903, the ratio $\frac{326254-315903}{354583-315903}$(=.267) must be less than .512.

For 356709, the ratio $\frac{356709-354583}{356709-326254}$(.069) is also below the critical value.

Thus all counts can be considered the same.[18]

Series 11

T.T. Peak and Biuret Analysis 20 mins/sample Bob I

Sample	Shear Rate (sec^{-1})	Shear Stress ($dynes/cm^2$)	Max Temp (°C)	MG%Fib (T.T.)	MG% (Biuret)	%Clot(T.T.)
1	0	0	22.0	285	302	100
2	24033 - 30105	495	26.5	239	302	85.6
3	47055 - 62487	1000	29.9	114	285	40.9
4	63246 -74630 - 70836	1253	31.4	192	310	68.8
5	68306 - 85003	1506	32.5	229	285	82.0
6	104989 - 95881	1988	35.4	239	302	85.6
7	107519 - 101447	2493	36.6	217	285	77.8
8	0	0	22.0	273	285	100

Series 12

T.T. Analysis 20 minutes/sample Bob I

Sample	Shear Rate (sec^{-1})	Shear Stress ($dynes/cm^2$)	MG%Fib	%Clot	Comments
1	0	0	327	100	control
2	4806	116	327	100	
3	7589	230	305	93.3	
4	21500	425	287	87.8	
5	47308 - 56516	770	260	79.5	
6	58692 - 70836	956	230	70.3	
7	73113 - 85000	1140	260	79.5	
8	0	0	327	100	control

Series 13

T.T. Peak Analysis 20 mins/sample Bob I

Sample	Shear Rate (sec^{-1})	Shear Stress ($dynes/cm^2$)	MG%Fib	%Clot	Comments
1	0	0	255(258)	100	control
2	42248-54392-76654	954	232	89.9	
3	56922-69575	977	186	72.1	
4	69065-77414	1080	238	92.2	
5	70580-79440	1138	226	87.6	
6	59960-69065	954	232	89.9	
7	61475-74880	1046	190	73.6	
8	0	0	262(258)	100	control

Series 14

T.T. Peak Analysis Bob I

Sample	Shear Rate (sec^{-1})	Shear Stress ($dynes/cm^2$)	Exposure (mins)	MG%Fib	%Clot	Comments
1	0	0	0	315	100	control
2	28080-32380	495	10	297	94.3	
3	28840-31120	495	25	310	98.4	
4	28590-30860	495	70	304	96.5	
5	28840-33650	495	55	285	90.5	
6	29360-33650	495	40	285	90.5	
7	0	0	0	260	82.5	control (?)

Series 15

T.T. Peak Analysis, 20 mins/sample Bob I

Sample	Shear Rate (sec^{-1})	Shear Stress ($dynes/cm^2$)	MG%Fib	%Clot	Comments
1	0	0	335(300)	100	control
2	70580-81970	1230	273	91	
3	62490-77920	1161	285	95	
4	64760-72350	1030	260	86.7	
5	52870-67290	931	285	95	
6	42000-63500	816	291	97	discard
7	47560-53130	816	291	97	
8	0	0	298(300)	100	control

Series 16

T.T. Peak Analysis Bob II

Sample	Shear Rate (sec^{-1})	Shear Stress ($dynes/cm^2$)	Exposure(mins)	MG%Fib	%Clot	Comments
1	0	0	0	310	100	control
2	24150-31770	497	40	273	88	
3	29040-20470	474	70	285	91.9	
4	29330-31050	497	25	297	95.8	
5	24010-30910	497	55	285	91.9	
6	0	0	0	285	91.9	control
7	0	0	0	310	100	control

Series 17

T.T. Peak Analysis Bob I

Sample	Shear Rate (sec^{-1})	Shear Stress ($dynes/cm^2$)	Exposure(mins)	MG%Fib	%Clot	Comments
1	0	0	0	310	100	control
2	62490-75900	1023	25	211	68	
3	55660-67800	990	55	211	68	
4	64760-74380-69820	990	70	124	40	
5	63250-71850-70580	990	40	260	84	
6	65780-70330	990	10	285	92	
7	64510-71340	990	25	260	84	
8	0	0	0	310	100	control

Series 18

T.T. Peak Analysis Bob I

Sample	Shear Rate (sec^{-1})	Shear Stress ($dynes/cm^2$)	Exposure(mins)	MG%Fib	%Clot	Comments
1	0	0	0	347	100	control
2	42500-56170	770	25	155	44.7	
3	44780-53640-48580	700	70	273	78.7	
4	42000-52120-50600	725	55	174	50.1	
5	46050-51100	725	40	260	74.9	
6	47560-50600	725	10	298	85.9	
7	0	0	0	347	100	control
8	0	0	0	347	100	control
9	0	0	0	347	100	control

Series 19

T.T. Peak Analysis Bob I

Sample	Shear Rate (sec^{-1})	Shear Stress ($dynes/cm^2$)	Exposure(mins)	MG%Fib	%Clot	Comments
1	0	0	0	297	100	control
2	43510-57430	793	40	248	83.5	
3	46800-56925	793	71	198	66.6	
4	47820-56670	793	55	186	62.6	
5	50850-56925	793	25	285	95.9	
6	47310-57180	793	25	285	95.9	
7	46550-55660	793	25	273	91.9	
8	0	0	0	297	100	control
9	0	0	0	297	100	control

Series 20

T.T. Peak Analysis 20 mins/sample Bob II

Sample	Shear Rate (sec^{-1})	Shear Stress ($dynes/cm^2$)	MG%Fib	%Clot*	Max Temp(^oC)	Comments
1	0	0	310			Group A
2	0	0	328			Group B
3	0	0	322			Group C
4	0	0	322	100		control
5	17830-25890	428	310	96.3	24.3	
6	29340-35090	636	322	100	27.0	
7	36670-37100	832	322	100	28.3	
8	39690-40270	1004	266	82.6	29.3	
9	42424-41560-	1120	285	88.5	30.1	
10	38110-37390-37680	889	322	100	28.8	
11	35230-34515	705	322	100	28.0	
12	25740-31500	497	291	90.4	27.0	
13	13230-16250	289	310	96.3	26.2	
14	24880-33080	566	310	96.3	27.2	
15	0	0	310	96.3	22.0	Control

*Analysis after freezing 5-15 for 3 days showed no difference in % clot.

Series 21

T.T. Peak Analysis Bob I

Sample	Shear Rate (sec^{-1})	Shear Stress ($dynes/cm^2$)	Exposure Time(mins)	MG%Fib	%Clot	Max Temp
1	0	0	control	260	100	22.0
2	15300-17600	288	25 mins exp.	260	100	23.0
3	13770-14280	288	55 mins exp.	248	95	23.5
4	14000-15300	288	42 mins exp.	260	100	23.5
5	15050-17850	288	70 mins exp.	242	93	24.0
6	15300-16200	288	10 mins exp.	260	100	24.0
7	0	0	control	260	100	22.0

Gel Analyses

The electrophoresis gels were analyzed by comparison of the areas under the alpha and gamma peaks to the area under the beta peak. The peak values were obtained with the microdensitometer analyses.

Sample	Shear Stress ($dynes/cm^2$)	Exposure (mins)	α/β	$\gamma dim/\beta$
		Series 5		
1	0	0	.20	.75
2	5217	20		
3	1988	40	.636	.727
4	1988	10	.222	.777
5	1988	20	.5	.625
6	0	0	.227	.818
		Series 6		
1	0	0	.132	.842
2	3228	10	.308	.666
3	3250	40	.447	.851
4	3228	30	.657	.714
5	0	0	.152	.818

Sample	Shear Stress (dynes/cm^2)	Exposure (mins)	α/β	γ_{dim}/β
		Series 8		
1(Bob I)	0	0	.364	.742
2	495	20	.50	.667
3	1000	20	.273	.655
4	1529	20	.448	.655
5	2011	20	.460	.698
6	2516	20	.446	.696
7(Bob II)	497	20	.167	.667
8	1005	20	.283	.761
9	2043	20	.5	.661
10	2528	20	.325	.675
11	0	0	.352	.648
		Series 11		
1	0	0	.167	.70
2	495	20	.493	.642
3	1000	20	.417	.719
4	1253	20	.662	.765
5	1506	20	.269	.596
6	1988	20	.578	.625
7	2493	20	.418	.527
8	0	0	.216	.635

Sample	Shear Stress (dynes/cm^2)	Exposure (mins)	α/β	γ_{dim}/β
		Series 15		
1	0	0	.143	.786
2	495	10	.321	.667
3	495	25	.333	.788
4	495	70	.298	.895
5	495	55	.373	.831
6	495	40	.241	.828
7	495	0	.435	.717
		Series 16		
1	0	0	.390	.719
2	1230	20	.476	.667
3	1161	20	.453	.688
4	1023	20	.578	.703
5	931	20	.448	.716
6	816	20	.211	.772
7	816	20	.300	.800
8	0	0	.333	.683

Sample	Shear Stress (dynes/cm^2)	Exposure (mins)	α/β	γ_{dim}/β
		Series 18		
1	0	0	.309	.629
2	1023	25	.609	.674
3	990	55	.781	.658
4	990	70	.676	.649
5	990	40		
6	990	10	.547	.629
7	990	25	.533	.653
8	0	0	.172	.644

Series 5

Sample	β	α	γdimer	α^1polymer	α^2polymer
1	20	4	15	3	6
2	0	0	0	0	0
3	33	21	24	9	9
4	9	2	7	1	2
5	8	4	5	-	-
6	22	5	18	1	1

Series 6

Sample	β	α	γdimer	α^1polymer	α^2polymer
1	38	5	32	0	1
2	39	12	26	0	2
3	47	21	40	1	2
4	70	46	50	5	4
5	66	10	54	4	5

Series 8

Sample	β	α	γdimer	α^1polymer	α^2polymer
(Bob I)					
1	66	24	49	5	11
2	66	33	44	11	11
3	55	15	36	3	5
4	58	26	38	4	7
5	63	29	44	7	7
6	56	25	39	7	4
(Bob II)					
7	54	9	36	2	4
8	46	13	35	1	1
9	56	28	37	4	3
10	40	13	27	6	5
11	54	19	35	2	3

Series 11

Sample	β	α	γdimer	α^1polymer	α^2polymer
1	60	10	42	4	4
2	67	33	43	9	9
3	96	40	69	11	19
4	68	45	52	11	12
5	52	14	31	5	8
6	64	37	40	2	2
7	55	23	29	3	5
8	74	16	47	5	5

Series 15

Sample	β	α	γdimer	α^1polymer	α^2polymer
1	70	10	55	3	8
2	81	26	54	5	7
3	66	22	52	15	16
4	57	17	51	11	9
5	59	22	49	1	3
6	58	14	48	21	13
7	92	40	66	13	18

Series 16

Sample	β	α	γdimer	α^1polymer	α^2polymer
1	64	25	46	7	11
2	63	30	42	4	10
3	64	20	44	4	7
4	64	37	45	4	7
5	67	30	48	7	8
6	57	12	44	3	5
7	50	15	40	4	3
8	60	20	41	2	5

Series 18

Sample	β	α	Ydimer	α^1polymer	α^2polymer
1	97	30	61	1	4
2	92	56	62	9	8
3	73	57	48	7	10
4	74	50	48	2	7
5	Bad Gel				
6	86	47	54	1	3
7	75	40	49	5	2
8	87	15	56	2	4

Series 4

(20 min Exposures)

Sample	Shear Stress (dynes/cm^2)	P.T. Peak	P.T.T. Peak
1	0	49	44
2	0	49	48
3	520	47.5	46.5
4	1000	47	47
5	3220	48	39
6	1988	47.5	49
7	0	47.5	45

Series 7

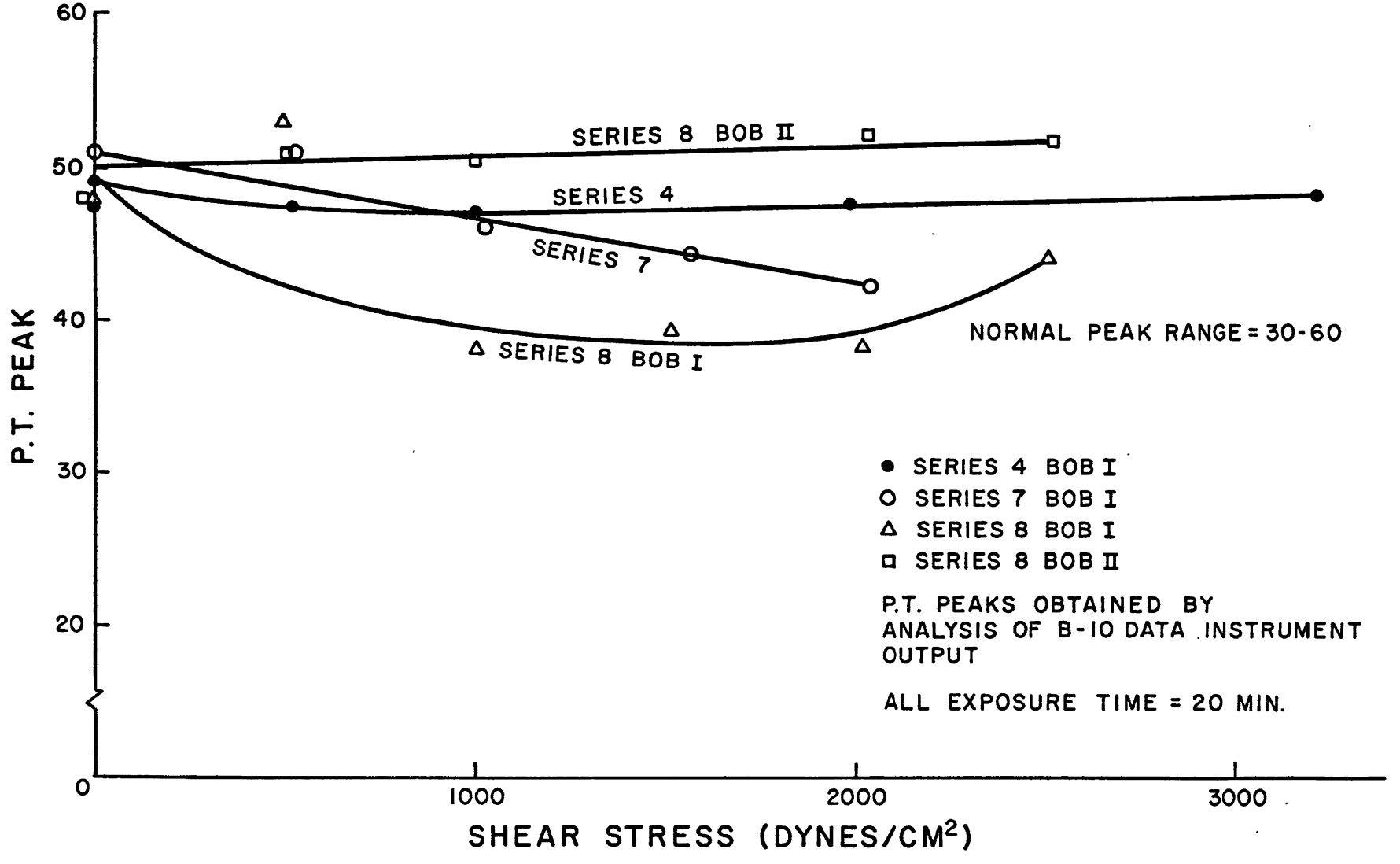
(20 min Exposures)

1	0	51	44
2	518	51	35.5
3	1023	46	36
4	2034	42	39
5	1574	44	38
6	0	49	47.5

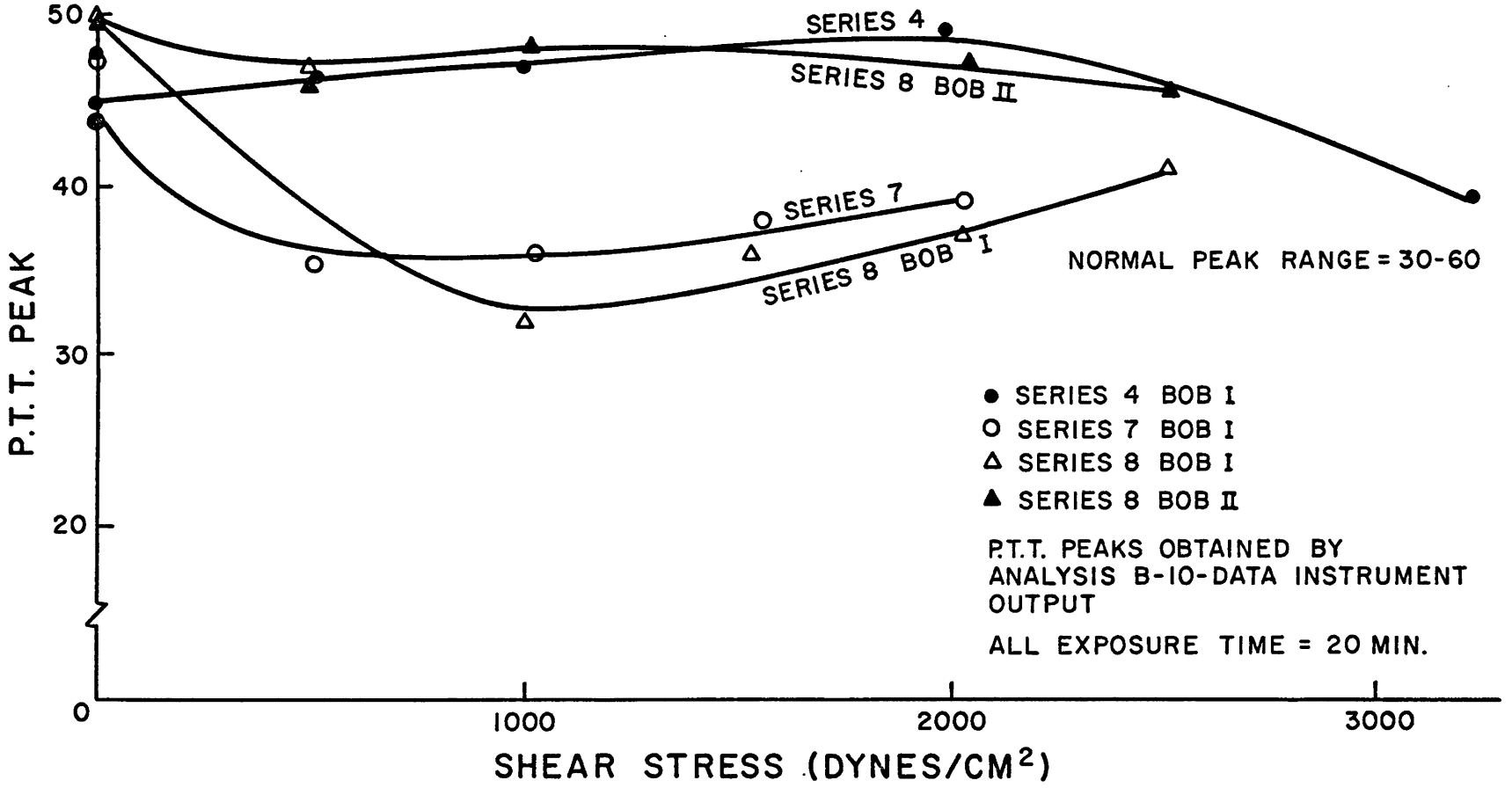
Series 8

(20 min exposures)

Sample	Shear Stress (dynes/cm^2)	P.T. Peak	P.T.T. Peak
1(Bob I)	0	48	50
2	495	53	47
3	1000	38	32
4	1529	39	36
5	2011	38	37
6	2516	44	41
7(Bob II)	497	51	46
8	1005	50	48
9	2043	52	47
10	2528	51.5	45.5
11	0	48	50



Prothrombin Time Versus Shear Stress



Partial Thromboplastin Time versus Shear Stress

APPENDIX IV

RAW DATA FOR SHEAR STUDIES

Series 1

Bob II 10 mins/sample Preliminary series, temps not recorded (Room temp = 21.2°C)

Sample	V_{rpm} (mv)	V_{torq} (mv)	V_{temp} (mv)	Start up (sec)	Stop (sec)	Comments
1	0	0		0	0	control
2	1-2	1		0	0	hand turned
3	200	23		5	5	
4	303	92-89		15	10	
5	492	140-133		25	11	

Series 2

Bob II 10 mins/sample Preliminary series, temps not recorded (Room temp = 21.2°C)

Sample	V_{rpm} (mv)	V_{torq} (mv)	V_{temp} (mv)	Start up (sec)	Stop (sec)	Comments
1	0	0	0	0	0	control
2	168-170	23	1080	15	5	
3	275	44	1250	15	7.5	
4	350	77	-	20	9	
5	402	99	1550	15	10	
6	500	142	1450	20	11	
7	450	121	1320	15	10	
8	620	195	1750	10	12	
9	0	0	930	0	0	sample placed in CSSV, not sheared

Series 3

Bob I

Sample	V_{rpm}	V_{torq}	V_{temp}^{max}	Start (sec)	Stop (sec)	Comments
1,4,5	500	159	1750	30	10	20 min run
2,8,14	0	0	0	0	0	control
3,9,11	505	159	1650	30	10	60 min run
6,7,15	0	0	1180	placed in CSSV 15 mins., not sheared		
10,12,13	0	0	0	0	0	control

Series 4

Bob I 20 minutes/sample

Sample	V_{rpm}	V_{torq}	V_{temp}^{max}	Start (sec)	Stop (sec)	Comments
1	0	0	0	0	0	control
2	0	0	0	plasma placed in CSSV, not sheared		
3	125-133	23	1160	8	5	
4	265-291	44	1370	12	6	
5	496-487-490	141	1610	10	8	
6	423-394	88	1360	30	7	turbulence problems
7	0	0	0	0	0	final control (4 hrs)

Series 5

Bob I

Sample	V_{rpm}	V_{torq}	V_{temp}^{max}	Start (sec)	Stop (sec)	Comments
1	0	0	0	0	0	control
2	740	240≠10	1900	45	11	20 mins.
3	413-391	87	1740	20	7	40 mins.
4	405-396	87	1380?	30	7	10 mins.
5	423-405	87	1380?	40	7	20 mins.
6	0	0	0	0	0	Final control (3 hrs)

Series 6

Bob I

Sample	V_{rpm}	V_{torq}	V_{temp}^{max}	Start (sec)	Stop (sec)	Comments
1	0	0	0			control
2	481-488	140	1750	30	8	approx. 10 mins.
3	489	140	1640	12	8	40 mins.
4	485-483	140	1620	20	9	30 mins.
5	0	0	0			control

Series 7

Bob I 20 minutes/sample

Sample	V_{rpm}	V_{torq}	V^{max}_{temp}	Start (sec)	Stop (sec)	Comments
1	0	0	0	0	0	control
2	121-127	22	1250	7	3	21 mins
3	266-305	44	1300	12	5	
4	430-400	89	1330	15	7	
5	374-330-390	68	1620	120	7	turbulence problem
6	0	0	0	0	0	control (3 hrs)

Series 8

Bob I 20 minutes/run

Sample	V_{rpm}	V_{torq}	V^{max}_{temp}	Start (sec)	Stop (sec)	Comments
1	0	0	0	0	0	control
2	106-118	22	1210	10	3	
3	223-249	44	1370	20	3	
4	375-355-385	66	1630	20	7	turbulence problems
5	415-400	88	1775	30	7	
6	440-412-419	110	1860	15	8	
7(BobII)	170-215	22	1285	15	5	
8 "	275-285	44	1480	20	6	
9 "	389-415	89	1780	12	8	
10 "	437-457	110	1860	15	9	
11	0	0	0	0	0	control

Series 9

Bob I Isotope Run (I^{125} labeled fibrinogen used) 20 min/sample

Sample	V_{rpm}	V_{torq}	V^{max}_{temp}	Start (sec)	Stop (sec)	Comments
1	0	0	0	0	0	control
2	0	0	0	0	0	control
3	108-121	22	890	15	3	
4	230-257	44	1325	10	5	
5	350-402-385	66≠ 5	1540	12	7	turbulence problems
6	440-408	89	1710	30	8	
7	463-430	110	1850	10	8	forced convex cooling

Series 10

Bob I 20 minutes/run Isotope run (I^{125} fibrinogen)

Sample	V_{rpm}	V_{torq}	V^{max}_{temp}	Start (sec)	Stop (sec)	Comments
1	0	0	0	0	0	control
2	125-127	21	1340	10	3	
3	250-311	45	1530	20	6	
4	387-405-370	66≠6	1725	15	7	turbulence problem
5	425-390	88≠8	1875	10	8	turb prob., cooling
6	456-435-431	109	1875	10	8	
7	0	0	0	0	0	control

Series 11

Bob I 20 minutes/sample

Sample	V_{rpm}	V_{torq}	V_{temp}^{max}	Start (sec)	Stop (sec)	Comments
1	0	0	0	0	0	control
2	95-119	22	1355	10	3	
3	186-247	44	1530	20	5	
4	250-295-280	55	1610	5	6	turbulence problem
5	270-336	66	1665	20	7	
6	415-379	87	1820	20	8	
7	425-401	109	1880	15	9	
8	0	0	0	0	0	Control

Series 12

Bob I 20 minutes/sample

Sample	V_{rpm}	V_{torq}	V_{temp}^{max}	Start (sec)	Stop (sec)	Comments
1	0	0	0	0	0	control
2	19	5	*	5	1	20.5 mins
3	29-30	10	*	10	1	
4	85	19	*	20	2	
5	187-223	34	*	20	3	
6	232-280	42	*	10	4	
7	289-336	50	*	10	5	
8	0	0	0	0	0	control

* Temperatures were not recorded due to previous evidence that no temperature probelms were encountered in this range of shear rates.

Series 13

Bob I 20 minutes/sample

Sample	V_{rpm}	V_{torq}	V_{temp}	Start (sec)	Stop (sec)	Comments
1	0	0	0	0	0	control
2	167-215-303	42	*	10	5	
3	225-275	43	*	15	4	
4	273-306	47	*	15	5	
5	279-314	50	*	15	5	
6	237-273	42	*	15	4	
7	243-296	46	*	15	5	
8	0	0	0	0	0	control

* See series 12

Series 14

Bob I

Sample	V_{rpm}	V_{torq}	V_{temp}	Start (sec)	Stop (sec)	Comments
1	0	0	0	0	0	control
2	111-128	22	*	10	3	10 mins
3	114-123	22	*	5	3	25 mins
4	112-122	22	*	10	3	70 mins
5	114-133	22	*	10	3	55 mins
6	116-133	22	*	10	3	40 mins
7	0	0	0	0	0	control

* See Series 12

Vibration caused average cup drop of .002 mm during each run

Series 15

Bob I, 20 minutes/sample

Sample	V_{rpm}	V_{torq}	V_{temp}	Start (sec)	Stop (sec)	Comments
1	0	0	0	0	0	control
2	279-330-315	54	*	20	5	
3	247-308	51	*	15	5	
4	256-286	45	*	15	5	
5	209-266	41	*	15	5	
6	257-166-251	36	*	15	5	cup slipped (discard)
7	188-210	36	*	15	4	
8	0	0	0	0	0	control

* See Series 12

Average cup drop of .006mm due to vibration

Series 16

Bob II

Sample	V_{rpm}	V_{torq}	V_{temp}	Start (sec)	Stop (sec)	Comments
1	0	0	0	0	0	control
2	168-221	22	*	5	4	40 mins
3	202-205	20	*	10	4	70 mins
4	204-216	22	*	10	4	25 mins
5	167-215	22	*	10	4	55 mins
6	0	0	0	0	0	control

* See Series 12

Series 17

Bob I

Sample	V_{rpm}	V_{torq}	V_{temp}	Start (sec)	Stop (sec)	Comments
1	0	0	0	0	0	control
2	247-300	45	*	10	5	25 mins
3	220-268	43	*	15	5	55 mins
4	256-294-276	43	*	10	5	70 mins
5	250-284-279	43	*	15	5	40 mins
6	280-278	43	*	10	5	10 mins
7	255-282	43	*	10	5	25 mins
8	0	0	0	0	0	control

* See Series 12

Average cup drop .005mm due to vibration

Series 18

Bob I

Sample	V_{rpm}	V_{torq}	V_{temp}	Start (sec)	Stop (sec)	Comments
1	0	0	0	0	0	control
2	168-222	34	*	10	4	25 mins
3	177-212-192	31	*	10	4	70 mins
4	166-206-200	32	*	10	4	55 mins
5	182-202	32	*	10	4	40 mins
6	188-200	32	*	5	4	10 mins
7,8,9	0	0	0	0	0	control

* See Series 12

Series 19

Bob I

Sample	V_{rpm}	V_{torq}	V_{temp}	Start (sec)	Stop (sec)	Comments
1	0	0	0	0	0	control
2	172-227	35	*	10	4	40 mins
3	185-225	35	*	10	4	71 mins
4	189-224	35	*	10	4	55 mins
5	201-225	35	*	10	4	25 mins
6	187-226	35	*	10	4	25 mins
7	184-220	35	*	10	4	25 mins
8	0	0	0	0	0	control

* See Series 12

Average cup drop due to vibration = 0.005mm

Series 20

Bob II 20 mins/sample

Sample	V_{rpm}	V_{torq}	V_{temp}	Start (sec)	Stop (sec)	Comments
1	Group A Control					
2	Group B Control		Three separate pools made to test consistency			
3	Group C Control					
4	Mixed Control (A+B+C)					Control
5	124-180	19	1240	10	4	
6	204-244-242	28	1380	10	5	
7	255-254-258	36	1445	10	5	
8	279-276-280	44	1500	10	6	
9	295-289-294	49	1540	15	6	
10	265-260-263	39	1475	20	5	
11	245-239-240	31	1435	20	5	
12	179-219-215	22	1380	10	4	
13	92-113	13	1340	10	3	
14	173-230	25	1390	10	5	
15	0	0	0	0	0	control

Series 21

Bob I

Sample	V_{rpm}	V_{torq}	V_{temp}^{max}	Start (sec)	Stop (sec)	Comments
1	0	0	0	0	0	control
2	60-69	13	1170	20	2	25 mins
3	54-56	12	1200	20	2	55 mins
4	55-60	13	1200	10	2	42 mins
5	59-70	13	1225	20	2	70 mins
6	60-64	13	1235	10	2	10 mins
7	0	0	0	0	0	control

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