Non-Newtonian Rheology of Human Blood - Effect of Fibrinogen Deduced by "Subtraction"

EDWARD W. MERRILL, GILES C. COKELET, ANTHONY BRITTEN and ROE E. WELLS, Jr.

Circ Res. 1963;13:48-55
doi: 10.1161/01.RES.13.1.48
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0009-7330. Online ISSN: 1524-4571

Non-Newtonian Rheology of Human Blood – Effect of Fibrinogen Deduced by "Subtraction"

By Edward W. Merrill, D.Sc., Giles C. Cokelet, D. Sc., Anthony Britten, M.D., and Roe E. Wells, Jr., M.D.

■ Blood has been recognized as a non-Newtonian fluid for many years. Only recently[1, 2] have the rheological properties of blood been studied under shear rates near zero flow. These studies have suggested that the viscosity of blood increases indefinitely as the shear rate approaches zero.

The problem of blood rheology under very low shear rates seemed to us worthy of further investigation, because in various vessels, or sequences of vessels, in the human microcirculation it is possible that, from time to time, blood flow may temporarily cease. The cinephotomicrographic studies of Fulton[4] and co-workers on the cheek pouch of the hamster demonstrate that intermittent cessation of flow of blood does in fact occur from time to time in healthy animals. If flow has completely ceased within a given sequence of vessels of the microcirculation, it is the rheological character of blood at zero shear rate that is relevant to the dynamics of re-starting flow.

Gabelnick[1, 3] was the first to compare the rheological characteristics of human blood at shear rates near zero flow with the characteristics of a suspension of red cells in Ringer's solution, at equivalent hematocrit levels and under equivalent shear rates. Gabelnick showed that the suspension of red cells in Ringer's solution was lower in viscosity by two orders of magnitude and more nearly Newtonian than blood. He concluded that elements present in plasma that are not present in Ringer's solution changed the *mechanism* of flow, and postulated interaction between the red cell surfaces and one or more of the proteins.

The experiments performed by Gabelnick directed attention to certain limitations of the original apparatus and of the experimental methods. Improvements in electronic instrumentation were made by Gilinson and Dauwalter.[6] The interpretation of experimental results of viscometric studies near zero shear proved to be far more complicated than one would have foreseen. Cokelet,[5] investigating the reasons for variations of the apparent viscosity of a blood sample with *time,* at constant low values of shear rate, showed that artifacts arise from "wall effects" in the viscometer. A layer rich in plasma, or perhaps consisting exclusively of plasma, forms in whole human blood under very slow ("creeping") flow, near zero flow. Thus, unless the experimental apparatus be designed to meet this condition, one measures the combined effects of viscous shear in two different media: (1) the plasma-rich wall layers, (2) the cell-rich main body of fluid. Studies of Cokelet led to redesign of the viscometer elements containing the blood sample, as described below. From these studies[5] it became clear that the rapidly increasing viscosity of blood as shear rate approached zero, noted previously,[1-3] signals a condition in which the fluid is approaching its yield stress (explained below). Consequently, as will be shown, it is more meaningful to describe the rheology of blood in terms of the yield stress and the shear stress-shear rate relation near zero shear rate, than in terms of viscosity. In a search for the elements responsible for yield

From the Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge; Massachusetts General Hospital and Peter Bent Brigham Hospital, Boston, Massachusetts.

Supported by U. S. Public Health Service Grants H-6423 and HTS-5500 from the National Heart Institute.

Received for publication January 18, 1963.

stress in blood, and related rheological characteristics, one avenue of approach is the "subtractive" method: beginning with whole blood, certain elements are removed and the result on rheological behavior is observed. For example, transferring red cells from plasma to Ringer's solution is tantamount to removing (more *or less* completely) all plasma proteins. Permitting plasma to clot, in order to obtain serum, is a "subtractive" procedure — in this case the subtracted element being almost exclusively fibrinogen. "Subtractive" experiments of this kind, and their interpretation, are the subject of this paper.

Experimental Methods

VISCOMETRY

A form of Couette Viscometer[6] was used for these studies (fig. 1), in which the shear stress and yield stress were detected as torques on outer cylinder *2,* while rotation of inner cylinder *1* at various speeds produced corresponding various values of shear rate in the blood. The lower part of figure 1 shows how the walls of the cylinders were vertically grooved, to a depth of 66 microns. Blood under test was contained in the annular space between these grooved walls and because of the relative roughness of the walls it was possible to minimize the effect of a plasma-rich wall layer and to interpret the experimental data in such a way that the rheological properties of blood as a homogeneous "continuum" (homogeneous only in the sense of uniformity of mechanical properties at all points) could be assessed. The methods being described elsewhere,[5] it is useful here only to note that, on account of the geometry of the cylinders the shear rate in inverse seconds at the inner surface of cylinder *2* was equal to 1.087 times the rotational speed of cylinder *1* in rev/min, and that the torque measured in dyne-cm on the inner surface at cylinder *2* was equal to 28.7 times the shear stress in the blood, measured in dyne/cm^2 at the inner surface at cylinder *2*. Temperature of the fluid samples in the viscometer were maintained at 25°C or 37°C by means of water forced down through tube *3* into cylinder *1,* made of coin silver, whence it returned to the water bath via tube *4* which also serves as the drive shaft for cylinder *1*. Cylinder *2,* being of lucite, was of such low thermal conductivity compared to silver as to act as insulator. Thus the fluid sample was forced to assume within ± 0.1°C the temperature of the circulating water. A guard ring *9* penetrated the surface of the blood in order to eliminate

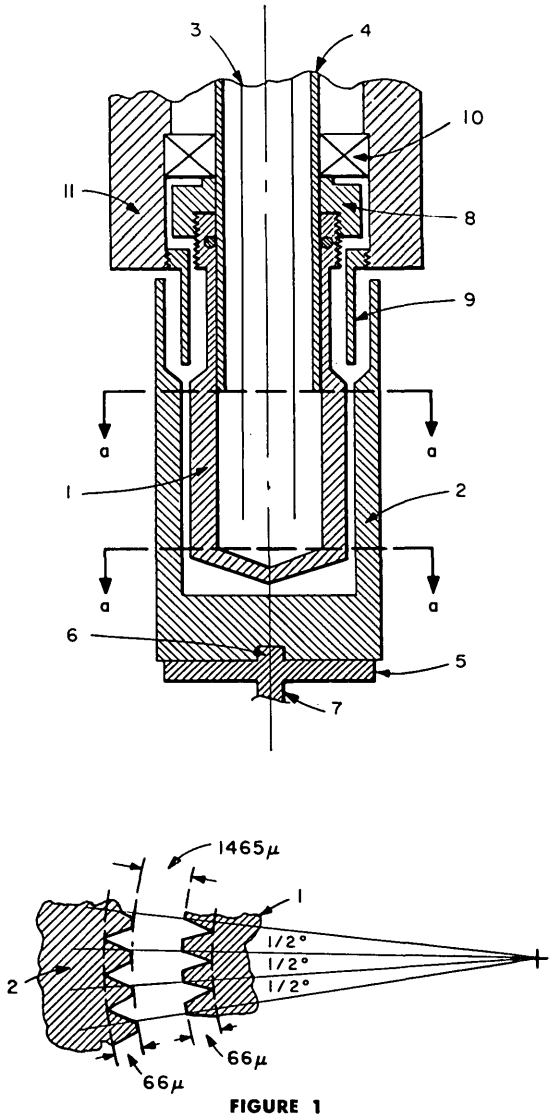

FIGURE 1

(Upper part) Cross section through axis showing coaxial cylinders and guard ring in relation to other parts of the viscometer. (Lower part) Cross section normal to axis at a-a, showing detail of grooves on inner and outer cylinders. (Figure reproduced with permission from Journal of Applied Physiology.)

the effects of denatured surface films that tend to form. Torque measurement is actually made electromagnetically[6] on shaft *7* which carries a table *5* on which the lucite cylinder *2* is centered, and locked, by stud *6*.

The viscometer consisting of the above described components was used to measure: (a) *the yield stress,* i.e., the maximum shearing stress that static blood as a homogeneous substance can support by elastic strain; or alternatively the minimum shear stress required to maintain the blood in viscous flow. Yield stress is the result of the spontaneous and usually reversible formation of a three-dimensional network of the particles suspended in a continuous medium when the suspension is at rest — a network that collapses under rather small stresses, turning the suspension again into a fluid. (b) *the dynamic rheological properties:* shear stress as a function of shear rate (rate of shear strain) under conditions of creeping flow near zero shear rate.

BLOOD AND SAMPLES PREPARED FROM BLOOD

Six experiments have been performed with blood drawn by conventional venopuncture, from six different donors in normal health in the Massachusetts General Hospital Blood Bank. While all the experiments lead to the same conclusions, to be discussed below, two are particularly illuminating and are described in detail. These are "subtractive" experiments in the sense that the beginning material was whole blood, from which specific elements were removed.

Experiment I

Most of the sample drawn from the donor was collected into a collection bag in which the amount of ACD (acid citrate dextrose) had been adjusted to give the normal concentration for administrable blood, but the last 50 ml was drawn into a siliconized tube contained in an ice bath. The contents of the siliconized tube were centrifuged at once (3°C), the supernatant plasma was drawn off and allowed to clot (25°C), and as soon as the clot had been removed (30 minutes) the serum thus produced was used as a suspending medium for red cells. These red cells were obtained by centrifugation from part of the original blood sample that had been drawn into ACD. The red cells were first "washed" by mixing and stirring them with an equal volume of serum, centrifuging, discarding the serum, and repeating this procedure. The red cells, thus twice "washed," were suspended in the serum at the desired hematocrit level.

Another portion of red cells was retrieved from the citrated whole blood, washed twice with Ringer's solution (equal volumes of cells and solution mixed, then centrifuged), and then suspended in Ringer's solution with a hematocrit level close to that of the red cell-serum and red cell-plasma suspensions.

A third portion of red cells, centrifuged from the citrated whole blood, was remixed with the plasma to give a suspension having a level of hematocrit close to the levels of the suspensions made with serum and with Ringer's solution. Experiments on the rheological properties of (i) the red cell-plasma suspension, (ii) the red cell-serum suspension, (iii) the red cell-Ringer's solution suspension, (iv) the clear plasma, (v) the clear serum, and (vi) Ringer's solution were then carried out at 25°C. Studies on the red cell-serum suspension were completed within two hours of the venopuncture, and the serum temperature never exceeded 25°C. Results of these rheological measurements are summarized in figure 2, to be discussed below.

Experiment II

The procedure was essentially the same as in experiment I except that in order to reduce the fibrinogen concentration of the serum to approximately zero, the serum was allowed to stand at 37°C for at least two hours.

The following suspensions of red cells were prepared:

(i) Red cells obtained from the whole blood sample by centrifugation were remixed with the original plasma containing ACD, so as to give a level of hematocrit of 43.1. Analysis of this plasma showed the fibrinogen concentration to be 0.23%.

(ii) A mixture of 50 volumes of plasma

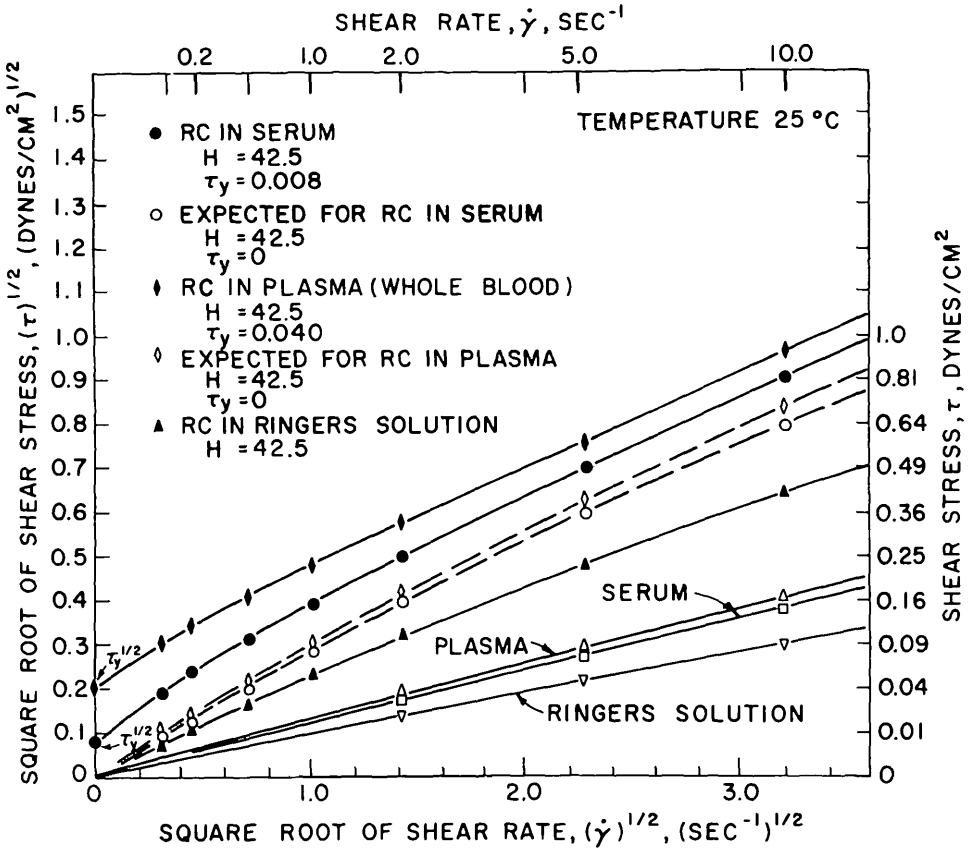

FIGURE 2

Square root of shear τ versus square root of shear rate $\dot{\gamma}$ for suspending media (plasma, serum, and Ringer's solution) and for suspensions of red cells in these media. H = hematocrit, τ_y = yield stress, dynes/cm².

and 50 volumes of serum that had been previously held at 37°C for nine hours to remove all thrombin activity was prepared. Red cells from the whole blood were washed twice with this mixture (equal volumes cells and solution), then suspended therein at the desired hematocrit. Analysis for fibrinogen in this solution showed 0.11%.

(iii) Serum, previously held at 37°C for two hours, was used first to wash the red cells (washing procedure as above), then to suspend the red cells at the desired level of hematocrit. Analysis for fibrinogen showed none detectable.

Rheological testing of the above suspensions and of the clear plasma and clear serum, was carried out at 37°C, and the results are summarized in figure 3.

Method of Presenting Data

The pertinent rheological features of non-Newtonian fluids can be presented in a variety of ways, and the selection of a method of presentation is, to a certain extent, arbitrary. For example, viscosity (a function of shear rate) can be plotted against shear rate or shear stress. Fluidity (reciprocal viscosity) can be similarly shown. Shear stress may be linearly plotted against shear rate, or log shear stress against log shear rate (if one seeks "power-law" constants). When the fluid possesses a yield stress, it is appropriate to focus attention on the primary variables shear stress and shear rate, rather than derived quantities such as viscosity.

We have chosen to present these primary variables as plots of the square root of shear

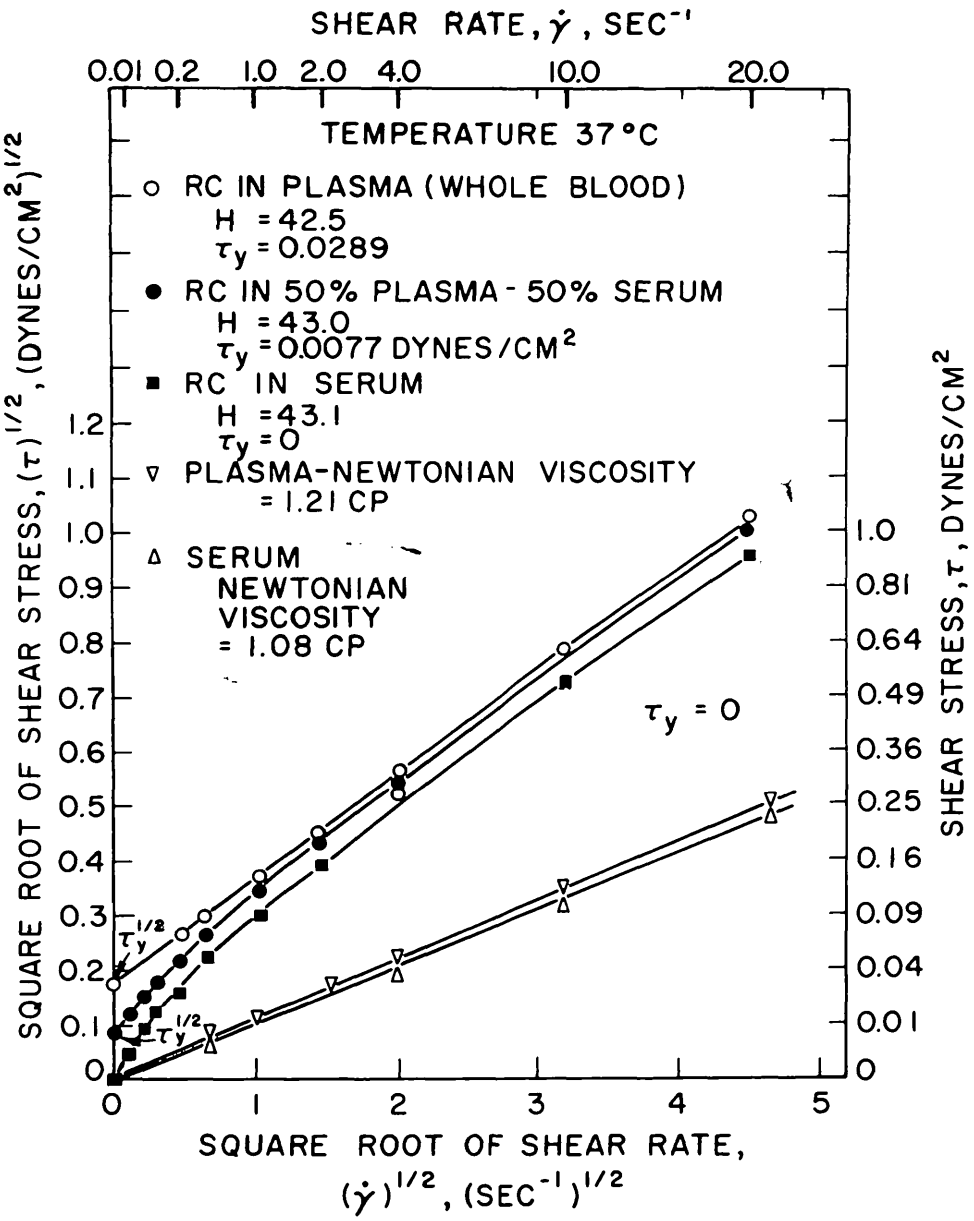

FIGURE 3

Square root of shear stress τ versus square root of shear rate $\dot{\gamma}$ for suspensions of red cells in plasma, in completely defibrinated serum, and in a mixture of these. Curves for plain serum and plain plasma are also shown. H = hematocrit, τ_y = yield stress, dynes/cm^2.

stress ($\tau^{1/2}$) against square root of shear rate ($\dot{\gamma}^{1/2}$) in order to compare the data thus plotted with Casson's equation:

$$\tau^{1/2} = \tau_y^{1/2} + b^{1/2} \cdot \dot{\gamma}^{1/2}$$

where τ_y = yield stress, b = constant for given system, its value depending in part on volume percentage dispersed phase, and viscosity of continuous medium.

The Casson plot is preferred to other possible methods of presentation of these original data first because of the possible importance of the physical model on which it was based

(to be discussed below). Secondly, from the viewpoint of concise presentation of the rheologically important features the Casson plot is more useful than direct curves of shear stress vs shear rate (yield stress is difficult to find), than curves of viscosity vs shear rate (viscosity goes off plot to infinity at zero shear rate), and than reciprocal viscosity vs shear stress (extrapolation to the yield stress at zero reciprocal viscosity is imprecise).

Results and Discussion

Experiment I (the results of which are summarized in figure 2) was designed in part to review the observations of Gabelnick[3] and to proceed further by comparing the effect of serum as a suspending medium relative to plasma.

In figure 2, one notes that each of the suspending media (Ringer's solution, plasma, and serum) is completely Newtonian over the range of interest, since $\tau^{1/2}$ is linear in $\dot{\gamma}^{1/2}$ and each curve passes through (0, 0). When the viscosity η, defined by

$$\tau = \eta\dot{\gamma}$$

is constant, the Casson relation must reduce to $\tau^{1/2} = b^{1/2}\ \dot{\gamma}^{1/2}$, $b = \eta$. Thus in figure 2 the slopes of the curves for Ringer's solutions, plasma, and serum represent the respective square roots of the *Newtonian* viscosities.

In an earlier publication, two of the present authors[8] reported that fresh plasma was non-Newtonian in this range of shear rate. This was later found to be an experimental artifact, caused by rigid layers of substances that spontaneously form at an air-plasma interface, probably including denatured plasma proteins. These artifacts are eliminated by use of the guard ring *9* of figure 1. Joly[9] confirms the necessity of using a guard ring in viscometric studies of plasma protein solutions.

The suspension of red cells in Ringer's solution at a level of hematocrit near the normal physiological value is slightly non-Newtonian, evident from the slight curvature shown in figure 2, but there is no yield stress whatever.

In contrast, the suspension of red cells in their original plasma at an equivalent level of hematocrit showed a yield stress and follows more or less closely the Casson equation. The suspension of the red cells in fresh serum showed a greatly reduced yield stress (as compared to red cells in plasma).

If there were no change in the mechanisms of flow in a suspension of particles as a consequence of changing only the continuous phase while leaving the volume fraction of dispersed particles unchanged, one would expect[10] that the shear stress-shear rate curves for the suspensions would be of the same shape, shifted relative to each other by the ratio of the viscosities of the continuous phase. Thus, for example, the curve shown as *calculated* for red cells in plasma (fig. 2) is obtained from the curve for red cells in Ringer's solution by increasing $\tau^{1/2}$, for each value of $\dot{\gamma}^{1/2}$, by a factor equal to the square root of the ratio of the Newtonian viscosities respectively of plasma and Ringer's solution. This procedure would give accurate results if one were concerned with the problem of predicting how the rheological characteristics of an emulsion of polystyrene would be altered by increasing the viscosity of the continuous phase. That such a procedure is completely wrong in the case at hand calls attention to the fact that some change in the *mechanism* of flow occurs, when red cells are transferred from plasma to Ringer's solution or vice versa, or from plasma to serum. Specifically, intercomparison of the curves of figure 2 suggested that fibrinogen had a predominant role in the non-Newtonian characteristics of blood. However the suspension of red cells in serum also showed a yield stress, though considerably less than that for the suspension of red cells in plasma. It was suspected that incomplete defibrination of the serum might have occurred.

In order to establish more clearly the role of fibrinogen, experiment II, described above, was performed in which quantitative analyses for fibrinogen were carried out. By holding the serum at 37°C for an extended period, it was cleared of fibrinogen, at least within the analytical limit of detection.

Figure 3 shows the results of experiment II. The principal additional finding is *complete absence* of yield stress in the suspension of red cells in serum. This appears to corroborate the conclusions drawn from experiment I, viz, that fibrinogen is a dominant factor in producing yield stress. In fact one could say that fibrinogen and whatever elements are precipitated with it in the clotting process are *sine qua non* factors in the creation of yield stress in normal human blood. Another part of experiment II indicates that the yield stress decreases as the fibrinogen content decreases, for the suspension of red cells in a mixture of half plasma, half serum, with a fibrinogen content about half that of the base plasma has a yield stress approximately one quarter of that found with the base plasma. Clearly the relationship of yield stress to fibrinogen content is non-linear.

As reported in detail elsewhere,[12] the yield stress in normal human blood is independent of temperature (at levels of hematocrit of 50 or less), and varies slightly from one subject to another according to an empirical relation of the form

$$\tau_y^{1/3} = C(H-H_c)$$

where H = actual hematocrit, H_c = critical hematocrit below which no yield stress occurs. C = constant for a particular blood sample, independent of ratio of red cells to plasma.

The critical value of H_c appears to lie between 1 and 5 volume per cent red cells; and the "constant" C varies from one normal subject to another by not more than ± 20%. Presumably C and H_c are largely, though not exclusively, determined by the natural fibrinogen content of the blood. In other experiments we have determined that the rheological characteristics of normal human blood are substantially identical, whether the blood has no anticoagulant, heparin as anticoagulant, or ACD solution as anticoagulant. Since the ACD *solution* in a collection bag somewhat dilutes the protein, whereas dry heparin produces no dilution, it was not surprising to find that the yield stress and the level of viscosity (as reflected by the slope b of the Casson equation) of blood drawn into ACD are slightly lower than the values for blood of the same hematocrit containing no anticoagulant, or heparin. Although heparin has some sequestering power for ionic calcium it is unlikely that it is as effective in sequestering calcium ion as ACD. In view of the almost identical yield stress values obtained, it appears that the interaction of fibrinogen with the red cells is not greatly affected by the level of ionic calcium.

The physical model on which Casson derived the equation cited above is that of reversible aggregation of the elementary suspended particles, through surface forces, into *rod-like* aggregates, which increase in length as shear stress decreases. Conversely, the rod-like aggregates under increasing stress reversibly decompose into smaller aggregates and ultimately into the elementary particles.

If one grants that because red cell-plasma suspensions obey (more or less) the Casson equation, they *may* follow the physical model underlying this equation, then one would presume the rod-like aggregates to be rouleaux.

The observations summarized in figures 2 and 3 show that fibrinogen and elements coprecipitated during defibrination are the only plasma elements that cause normal human blood to form spontaneously at rest a three-dimensional structure of which the yield stress is a manifestation. In the light of Fåhraeus' exposition[11] of the importance of fibrinogen to rouleaux formation, it is an easy, though not necessarily correct, step to assume that this three-dimensional structure is built out of preorganized rouleaux. One can unequivocably decide that those elements remaining in serum after defibrination, viz. albumin, α-globulin, and γ-globulin do not cause a yield stress in suspensions of red cells. Not clear, however, are the roles of other elements such as plasminogen, thrombin, antihemophilic globulin, and fibrinase, that are precipitated in the process of fibrinogen transformation to fibrin. Nor is it known with what sites on the red cell surface the fibrinogen interacts or how the fibrinogen molecule is aligned with respect to the cell membrane.

It seems evident that further studies are necessary, both to establish the biophysical nature of the red cell-plasma structure in the static state and under low shear rates, and to ascertain the physiological relevance of the peculiar rheological properties resulting from the red cell-plasma interaction.

Summary

A study of the rheological properties of human blood, from donors in normal health, was carried out by means of a coaxial cylinder viscometer designed to measure very small levels of stress under conditions of "creeping" flow.

It was found that under these conditions of measurement the rheological properties could be conveniently presented by plotting the square root of shear stress against square root of shear rate. For normal blood, a nearly linear relation is found on such a plot, and the intercept on the stress axis at zero shear rate represents the square root of yield stress, separate determination of which is made by other means.

Similar plots for (i) defibrinated blood and (ii) suspensions of red cells in isotonic saline solution reveal no yield stress. Thus it is concluded that fibrinogen is essential for the existence of yield stress in human blood. Furthermore, the approximate linearity, for normal blood, of the square root of shear stress with square root of shear rate, and the yield stress intercept, are of great interest inasmuch as mathematically identical relations ensue according to an equation developed by Casson based on a physical model in which the elementary particles of a suspension are capable of reversible association into rod-like structures, the length of which is controlled by the shear rate. It is of interest to consider the Casson model in the light of rouleaux formation and the relation of fibrinogen to rouleaux formation.

Acknowledgment

We wish to note our gratitude to Professor C. Stark Draper, Head of the Instrumentation Laboratory at M.I.T. for encouraging the building of the viscometer and for making it available to the M.I.T. Blood Rheology Laboratory; to P. J. Gilinson and C. R. Dauwalter of the M.I.T. Instrumentation Laboratory for their design of and continuing work on the viscometer; to M. Grove-Rasmussen, M.D. and R. Shaw, M.D., director and associate director of the Blood Bank of the Massachusetts General Hospital, for their generous cooperation and invaluable advice.

References

1. Wells, R. E., Jr., Merrill, E. W., and Gabelnick, H.: Shear rate dependence of viscosity of blood: Interaction of red cells and plasma proteins. Trans. Soc. Rheol. 6: 19, 1962.
2. Dintenfass, L.: Thixtotropy of blood and proneness to thrombus formation, Circulation Res. 11: 233, 1962.
3. Gabelnick, H.: M.I.T. Blood Rheology Laboratory, Private Communication to E. W. Merrill, Aug. 31, 1961.
4. Fulton, G., and Lutz, B. R.: Kinemicrography of living blood vessels. Med. Biol. Illustration 7: 26, 1957.
5. Cokelet, G., Merrill, E. W., Gilliland, E. R., Britten, A., and Wells, R. E.: Rheology of human blood-measurement near and at zero shear rate, presented at Annual Meeting of Society of Rheology (American Institute of Physics), Baltimore, October, 1962. Accepted for publication Trans. Soc. Rheol. 7: 1963.
6. Gilinson, P. J., Jr., Dauwalter, C. R., and Merrill, E. W.: Rotational viscometer using an A. C. torque-to-balance loop and an air bearing, presented at Annual Meeting of Society of Rheology (American Institute of Physics), Baltimore, October, 1962. Accepted for publication Trans. Soc. Rheol. 7: 1963.
7. Casson, N.: Flow equation for pigment-oil suspensions of the printing ink type. chap. 5 *In* Rheology of Disperse Systems, ed. by C. C. Mills. New York, Pergamon Press, 1959.
8. Wells, R. E., and Merrill, E. W.: Shear rate dependence of the viscosity of whole blood and plasma. Science 133: 763, 1961.
9. Joly, M.: Dispositif pour la viscosimetre precise de systèmes contenant des proteines. Biorheology 1: 15, 1962.
10. Krieger, I. M., and Dougherty, T. J.: A mechanism for non-Newtonian flow in suspensions of rigid spheres. Trans. Soc. Rheol. 3: 137, 1959.
11. Fåhraeus, R.: Influence of the rouleau formation of erythrocytes on the rheology of the blood. Acta Med. Scand. 161: 151, 1958.
12. Merrill, E. W., Gilliland, E. R., Cokelet, G., Shin, H., Britten, A., and Wells, R. E., Jr.: Rheology of human blood, near and at zero flow—Effects of temperature and hematocrit. Accepted for publication Biophy. J.