RHEOLOGY OF HUMAN BLOOD, NEAR AND AT ZERO FLOW

EFFECTS OF TEMPERATURE AND HEMATOCRIT LEVEL

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ABSTRACT Static normal human blood possesses a distinctive yield stress. When the yield stress is exceeded, the same blood has a stress–shear rate function under creeping flow conditions closely following Casson's model, which implies reversible aggregation of red cells in rouleaux and flow dominated by movement of rouleaux. The yield stress is essentially independent of temperature and its cube root varies linearly with hematocrit value. The dynamic rheological properties in the creeping flow range are such that the relative viscosity of blood to water is almost independent of temperature. Questions raised by these data are discussed, including red cell aggregation promoted by elements in the plasma.

INTRODUCTION

Scope of this Work. In the studies reported herein normal human blood was examined as a *homogeneous medium*[1] for two rheological properties: (*a*) the strength in shear of stationary blood as an elastic solid (the *yield stress*), and (*b*) the shear stress function of shear rate, from the yield stress (zero shear rate) up to shear rates of 100 sec.$^{-1}$, with particular attention to this function between zero and 1.0 sec.$^{-1}$. No prior studies of the yield stress of static blood by direct measurement can be found in the literature, nor any studies of the dynamic rheology in this range of "creeping" flow (0 to 1 sec.$^{-1}$) under conditions such that the blood responded as a *homogeneous*[1] medium.

The yield stress of static blood and the dynamic rheological properties of blood under creeping shear were determined as functions of temperature and of red cell

[1] Homogeneous in *mechanical* properties, in a macroscopic frame of reference. Further definition of this term will be found in connection with *wall effects,* to be discussed later.

concentration. These data appear to afford some new information pertaining to the interaction between red cells that may be of interest to the biophysicist.

Rheological Definitions. The concept of yield stress is illustrated in Fig. 1. If a cube (Fig. 1*a*) of homogeneous elastic solid of unit face area, oriented in the coordinate system x, y, z as shown, experiences a shearing force in the x direction of magnitude F_x (corresponding to stress per unit area τ) an equilibrium

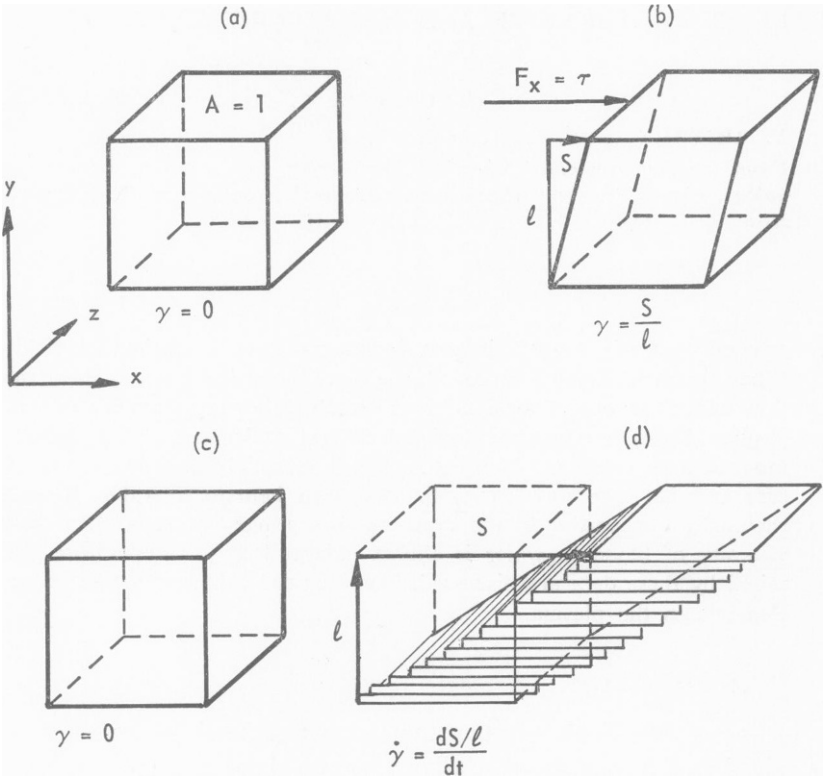

FIGURE 1 Deformation of a unit cube (*a*) of substance producing elastic strain (*b*), when stress applied is less than yield stress, followed by restoration to original shape upon removal of stress (*c*), or viscous shear (*d*) when stress applied exceeds the yield stress.

value of strain γ will be produced of magnitude s/l (Fig. 1*b*). If the stress is removed, the strain will return to zero (Fig. 1*c*). The ratio of stress to strain is the shear modulus. If a cube of homogeneous *fluid* (Newtonian or non-Newtonian) experiences such a stress, continuous viscous deformation will occur by slippage of differentially thin lamellar elements of the fluid (illustrated schematically by the plates of finite thickness in Fig. 1*d*). An equilibrium value of the rate of strain (the shear rate) will be established in response to the stress τ. The shear rate (the

rate of strain) is given by $\dot{\gamma} = ds/ldt$ (*v.* Fig. 1*d*). The ratio of stress to shear rate, $\tau/\dot{\gamma}$ is by definition the viscosity η.

In *plastic* fluids, of which blood is one example, when the applied stress is less than the *yield stress,* only elastic strain will ensue in the material. But when the stress exceeds the yield stress, continuous viscous flow will occur. Thus the yield stress τ_y is that value of τ which corresponds to transition between elastic deformation (Fig. 1*b*) and viscous flow (Fig. 1*d*).

It is commonly noted that the dynamic rheology of plastic fluids (*i.e.* what happens at stresses exceeding τ_y) follows a relation schematically indicated by Fig. 2*a*.

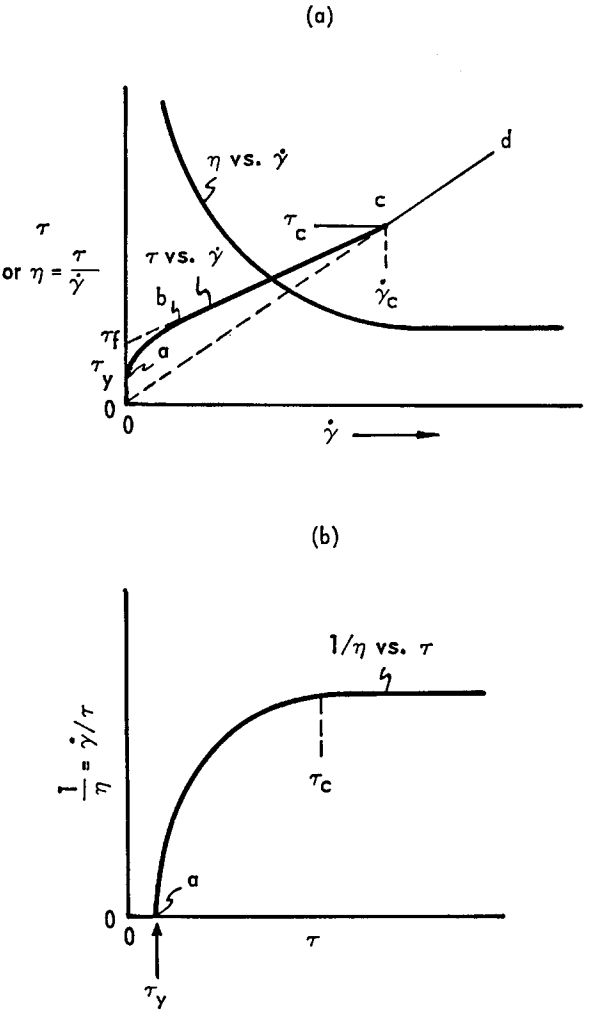

FIGURE 2 (*a*) Shear stress τ and viscosity $\eta(\eta = \tau/\dot{\gamma})$ as functions of shear rate $\dot{\gamma}$ for typical plastic fluid. See text for discussion. (*b*) Fluidity $(1/\eta)$ as a function of shear stress τ, replotted from Fig. 2*a*.

When stress τ is plotted *versus* shear rate $\dot{\gamma}$, after the yield stress (*a*) is exceeded, a curved relation *ab* is followed, leading to a straight section *bc*. If *bc* is extrapolated to zero $\dot{\gamma}$ an intercept τ_f is discovered which usually exceeds τ_y, the true yield stress. (In the ideal Bingham plastic $\tau_f = \tau_y$). Ultimately the τ *versus* $\dot{\gamma}$ relation must follow a straight line extrapolating through the origin (*cd* in Fig. 2*a*), which is *Newtonian* flow. The shear rate $\dot{\gamma}_c$ at which this occurs is widely variable in order of magnitude, generally lying between 10^2 and 10^5 sec.$^{-1}$. The shear rate function of viscosity η corresponding to the τ *versus* $\dot{\gamma}$ function is shown for comparison in Fig. 2*a*. Above $\dot{\gamma}_c$, η becomes independent of shear rate (Newtonian flow). A disadvantage of the shear-rate function of viscosity is that it goes to infinity as $\dot{\gamma}$ approaches zero. Consequently it is often convenient to express the dynamic rheology of plastic fluids as the stress function of fluidity, fluidity defined as reciprocal viscosity. Such a plot is shown as Fig. 2*b*. At stresses exceeding τ_c the fluidity must, of course, be constant. A more advantageous method of expressing the dynamic rheology of blood, as the square root of stress *versus* the square root of shear rate, will be described below.

Physical Origins of Yield Stress. As has been noted elsewhere, (1, 2, 5) yield stress is a manifestation of attractive forces between particulate matter suspended in a continuous medium—forces operative in such a manner as to permit rapid locking of the particles into three-dimensional networks upon cessation of flow but weak enough so that the network will disintegrate (reversibly) under sufficient stress so as to permit viscous flow to occur. In general, one can identify ion bridges, hydrogen bonds, electrostatic charge, or in some cases simply Van der Waals fields as the origin of the locking forces between particles. In the case at hand, one is interested in the very substantial forces of attraction between the red cells.

Problem of Measuring Homogeneous Rheological Properties. One desires to determine the yield stress and the stress-shear rate relations of a dispersion of particulate elements in a continuous medium in such a manner that the dispersion responds as a mechanically *homogeneous* substance. In such a disperse system under flow, there are, at the walls of a confining vessel or viscometer, fewer particles than in the bulk liquid because of the "excluded volume" effect (Vand, 4). Hence there exists at the wall a "slip" film of lower viscosity than that of the bulk liquid. This effect becomes especially important when the boundary walls are separated by a distance less than 20-fold the average diameter of particles in the contained disperse substance, and when the walls are at the same time smooth, *i.e.* when the peak-to-valley roughness is, say, 1/50 or less of the disperse phase particle diameter (1). At low shear rates and at zero shear, organization of the elementary dispersed particles into clusters may occur and thereby magnify the Vand excluded volume effect, because the cluster dimension is very much greater than the dimension of the individual element. An even greater wall effect will occur if the elemen-

tary particles associate into a continuous network that can progressively densify itself (*via* Brownian agitation or as a consequence of orientation under shear) with the expression of substantial layers of the *clear* suspending medium next to the wall. This latter effect is also called syneresis.

It is difficult to design a capillary viscometer to obviate these wall effects, and if they could be obviated (by making a "rough" wall), one is still faced with the problem of resolving very small shear stresses (as very small pressure gradients) that are necessary if one desires to measure yield stress and dynamic rheology under creeping conditions. For these reasons, we have resorted to a form of Couette viscometer with grooved walls, in which it is possible to minimize the wall effects and to resolve the very small stresses that are found in creeping flow.

METHOD

Blood Sampling. All samples of blood were obtained from donors from the Massachusetts General Hospital Blood Bank. On account of the proximity of M.I.T. to the hospital, it was possible to commence rheological testing on a blood sample (contained in a siliconized tube stored in ice) 15 minutes after donation. A large number of previous tests convinced the authors that the key rheological features are substantially identical whether the sample be tested (immediately) without anticoagulant, or with normal amounts of heparin as the anticoagulant *if the testing be completed within 8 hours,* or with the normal amount of acid-citrate-dextrose (ACD). The plasma of blood drawn into transfusion bags containing ACD solution is diluted by the ACD solution so that even when the red cells and plasma phase, previously separated by centrifugation, are recombined to yield the original hematocrit reading, the concentration of plasma proteins is about 85 per cent of that in the *in vivo* plasma. Consequently the rheological properties of the ACD-treated blood (*viz.* the yield stress and the stress at a given shear rate) are slightly diminished as compared to heparinized blood, but no essential property is lost. As the stability of blood in ACD is so superior to its stability in heparin, we prefer to use ACD in all testing. Micro-clots, about the size of a pinhead, are found to develop in many samples of normal heparinized human blood within 12 hours of drawing. Although these clots may be removed, one suspects that the residual sample may be indefinitely altered in its rheological characteristics.

In general, exposure of the blood samples to air was minimized, so that the rheological properties obtained were (more or less) those corresponding to the composition of venous blood in respect to pH, p_{CO_2}, and p_{O_2}. Some variation in the values of p_{CO_2} by our method of procedure doubtless occurred. In one series of experiments, a sample was equilibrated under 20 per cent CO_2, 80 per cent N_2. Yield stress was slightly increased over that of the sample not so equilibrated, but no major change of rheological features was noted. In order to prepare samples of varying hematocrit values from the same whole blood, a refrigerated centrifuge was used. Separation of plasma from red cells was accomplished by spinning at 5000 RPM for 20 minutes at 3°C. Usually the buffy coat (white cells) was removed by aspiration. The red cells and plasma were recombined in various ratios, and the hematocrit level was determined by Wintrobe tubes centrifuged at 5000 RPM to equilibrium readings.

Viscometer. A form of Couette viscometer, designated GDM(6), was used

for these studies, as its ability to resolve very small shear stresses has proven essential for the accurate direct determination of the yield stress and for the low-shear dynamic rheology. The blood sample, about 8 ml, is contained in a plastic (polymethyl methacrylate) cup *2* of Fig. 3, which serves as the stationary cylinder. Rotating cylinder *1*, made of coin silver with siliconized surface, is driven at various speeds (0.02 RPM to 100 RPM) by shaft *4*, and is supplied internally with temperature-controlling water

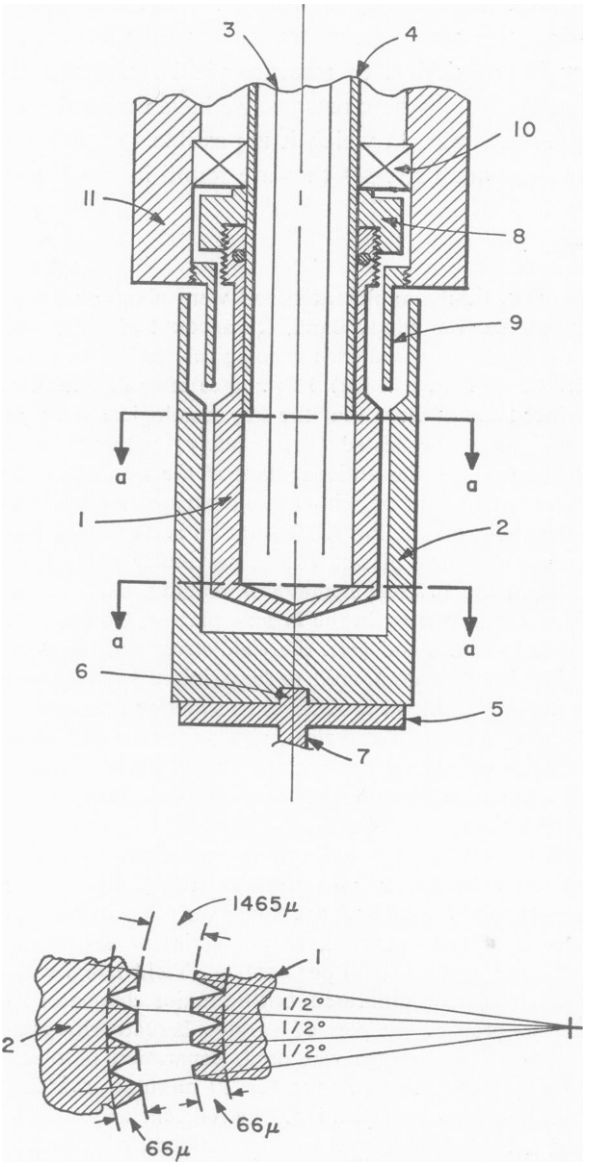

FIGURE 3 (above) Cross-section of coaxial cylinder assembly in GDM viscometer. (below) Detail of grooves in rotor and stator cylinders at section *a a*.

through tube *3*. The water returns through hollow shaft *4* into the water bath through a sealed manifold (not shown). Because of the high thermal conductivity of silver and its high volumetric heat capacity, compared to very low values of these properties for the plastic cup, the blood sample is maintained within 0.1°C at the temperature of the water circulating through the rotor.

To make a test, blood is placed in the cup *2* which is then mounted on torque-measuring table *5* centered by stud *6*. A clear plastic shield (not shown) is then fitted around the cup which also serves as a means for accurately locating the rotor, coaxially and vertically. The rotor assembly then is lowered into the cup, guided by the plastic shield. A guard ring (9), attached rigidly to the frame (11), intercepts the surface of blood so as to nullify the effect of rigid surface films that form between the guard ring and the cup, since, even though these films be semi-rigid, they undergo negligible strain and therefore contribute negligible torque. Coaxial grooves were cut in the surfaces of both cylinders as shown in Fig. 3. The depth of the V-groove (approximately 66 μ) is such as to make the cylindrical walls act "rough" to the blood. The depth of the groove is about 8 red cell diameters and probably comparable to the length of rouleaux. Thus the wall effects are minimized, because the plasma-rich phase cannot effectively operate in such a manner as to produce a torque-reducing slip film, which occurs against smooth walls.

Measurements of Stress and Yield Stress. Absolute measurement of yield stress in the sample is accomplished as indicated in Fig. 4, a typical recorder plot of torque *versus*

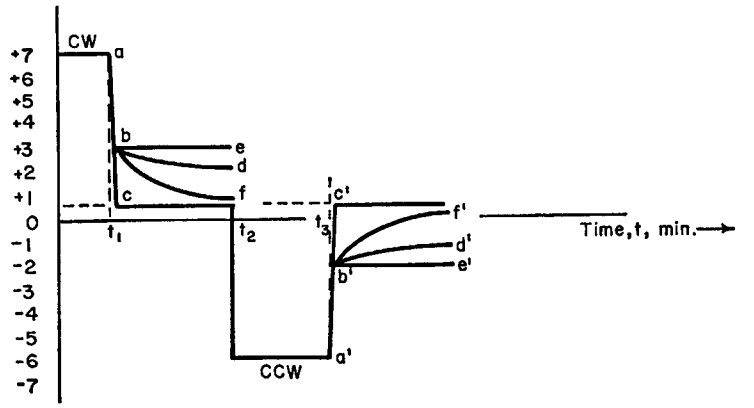

FIGURE 4 Typical record of torque for clockwise (CW) and counterclockwise (CCW) rotation *versus* time in yield stress tests. For interpretation see text.

time. After a period of clockwise rotation (CW) the rotor is suddenly stopped at time t_1 and if the liquid under test has *no* yield value (*e.g.* water, oil), the torque follows line *abc* to true zero (not necessarily the recorder zero) within 2 seconds. Counterclockwise rotation (CCW) at the same rotational speed begun at time t_2 produces a negative torque. Rotation is abruptly stopped at time t_3 and the torque signal decays rapidly to zero along *a'b'c'*. The arithmetic mean of *a* and *a'* give the absolute torque corresponding to the fixed RPM imposed before t_1 and t_3. Two sets of cylinders were used. Because of the geometry of these respective sets of cylinders, the shear stress τ, dynes/cm^2, is related to the thus-measured torque T, dyne-cm, by the relation $\tau = T/28.75$, or $\tau =$

$T/27.26$. The relations between the shear rate γ, sec.$^{-1}$, and the rotational speed N, RPM, were respectively: $\gamma = 0.74N$, or $\gamma = 1.02N$. If the liquid under test has a yield stress, then upon stopping rotation completely different curves are followed: for most human blood the shape is *abd*(CW) and *a'b'd'*(CCW) but for some samples an abrupt plateau *be* or *b'e'* is followed. The yield torque is measured as the arithmetic mean of *b* and *b'*, thereby eliminating zero error. In the usual experiment the torque, after *b* or *b'*, lethargically drifts toward zero over many minutes. It is quite certain that this torque decay is an artifact ascribable to the second heterophase effect (3), for if smooth (grooveless) cylinders of identical radii be used the torque decay in the *same* blood sample will follow curves *abf* or *a'b'f'*. In other words the torque will fairly promptly go to zero, whereas the decay is much slower with the grooved cylinders. That it occurs *at all* with some blood samples between grooved cylinders is a reflection, we believe, of strong syneresis, causing cleavage of the structure within the blood such that plasma layers form between the annulus of blood contained between the cylinders and the blood held in the grooves, so that the latter become isolated rods of triangular cross-section.

After a sample of blood has been placed in the cup and the viscometer has been assembled, the surrounding environment becomes filled with nitrogen due to the effiux of this gas from the gas bearing which causes table *5* to float frictionlessly. Without disturbing the sample, the temperature can be changed easily by regulating the temperature of water circulated through rotor *1*. In samples with a high sedimentation rate it was necessary periodically to "stir" the sample by alternately raising rotor *1* about 1 cm and then returning it to its designed position several times in succession.

RESULTS

The basic data (shear stress *versus* shear rate) are shown for a typical sample of blood, treated with ACD, from a normal donor (male) in Fig. 5. As mentioned previously these curves are substantially identical in shape with those for the same

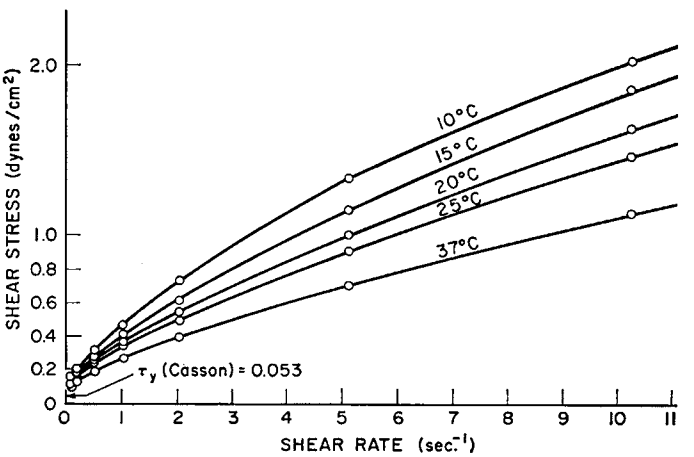

FIGURE 5 Shear stress τ *versus* shear rate γ for human blood from a male donor, containing ACD as anticoagulant, at constant hematocrit of 44.8. Temperature of test indicated against each curve. Note that the extrapolated intercepts on the stress axis exceed the true yield stress (0.053 dynes/cm²).

blood with heparin or without any anticoagulant. The levels of stress are perhaps 5 to 10 per cent lower.

These data may be transcribed to show viscosity *versus* shear rate or fluidity *versus* shear stress (Fig. 2), and in fact calculation of viscosity ($\eta \equiv \tau/\dot{\gamma}$) as a function of shear rate is a precursor to Figs. 7 and 8, to be discussed below. In our opinion, the most fruitful treatment (3) of the data is illustrated in the Casson (7) plot, Fig. 6, which presents the square root of shear stress *versus* square root of

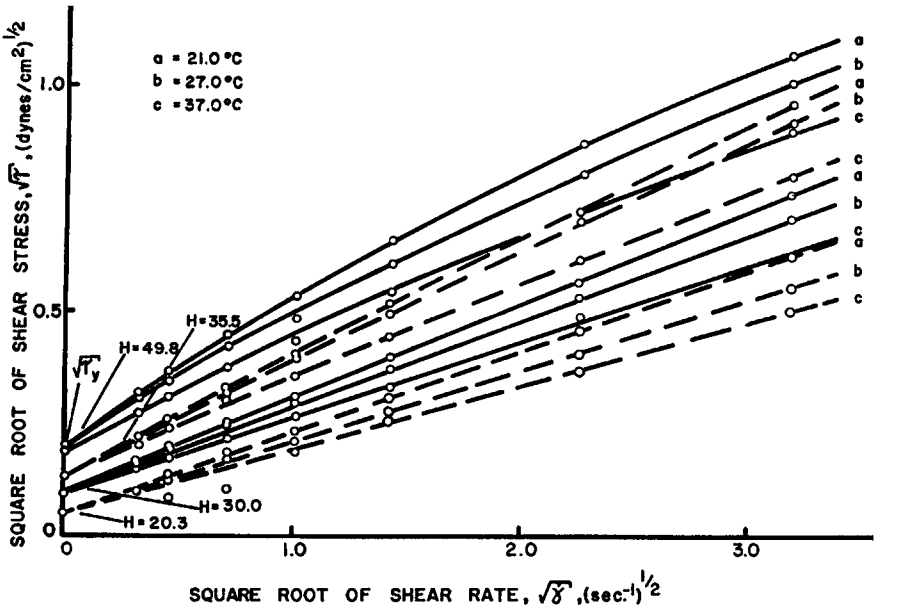

FIGURE 6 Casson plots (square root of shear stress *versus* square root of shear rate) for a typical nomal human blood, ACD as anticoagulant, at three temperatures, as indicated, for each of 4 hematocrit levels, as indicated.

shear rate, for four samples of the same red cells and plasma, from the same donor (not that of the sample of Fig. 5), made up in different hematocrit levels covering a clinically important range. The yield stress τ_y measured by the absolute method described above is indicated for each sample; *i.e.* the indicated value τ_y is *not* extrapolated.

In the first place, if a linear relation *not* extrapolating through the origin is found on such a plot, then the substance may follow the physical model proposed by Casson (7) for a special kind of plastic fluid flow. This model concerns particles which, as stress is reduced, form progressively longer *rod-like* aggregates through specific surface forces on the elementary particles. Conversely, as stress increases, the rod-like aggregates are increasingly dispersed into smaller aggregates and finally

into the elementary particles. Casson (7) found that his theoretical equation for this model:

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

(where b = constant dependent on particle type and viscosity of medium) well fitted the data on suspensions of certain particles in lithographic varnish.

It is evident from Fig. 6 that the Casson equation perfectly fits the data on blood at hematocrit levels of 20 and 30 (and at 21°, 27° and 37°C). At and above a hematocrit level of 35, some curvature is found, becoming greater the higher the hematocrit level, but extrapolation of the slight curves to the (stress) $^{1/2}$ axis gives an intercept that closely agrees with the value obtained by absolute measurement as described above. The value of τ_y from the Casson plot and the value obtained by absolute measurement, indicated against the curves for each sample, agree within 10 per cent, and the order is random—neither is consistently higher than the other.

Particularly noteworthy from Fig. 6 is the constancy of yield stress at a given

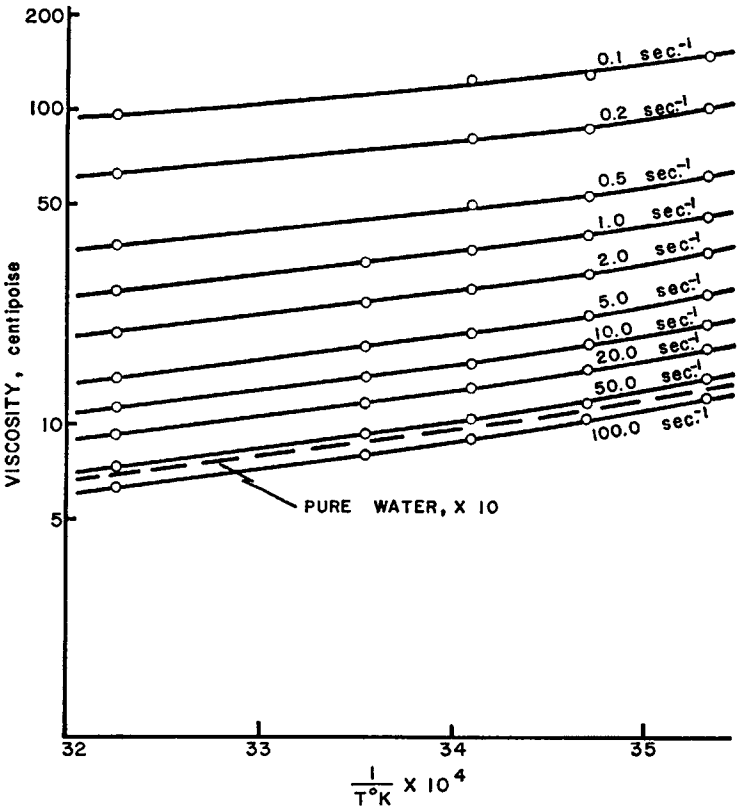

FIGURE 7 Viscosity (log scale) *versus* reciprocal absolute temperature determined from data of Fig. 5, at constant hematocrit level of 44.8 (ACD as anticoagulant), for shear rates between 0.1 and 100 sec.$^{-1}$ as indicated.

hematocrit level, independent of temperature, through a hematocrit value of 35. At higher hematocrit values, there is an increasing, but very weak, trend for the yield stress to increase as temperature drops.

In Fig. 7, the shear-dependent viscosity (ratio of shear stress to shear rate), as calculated from the shear stress *versus* shear rate data of Fig. 5, has been plotted logarithmically against reciprocal absolute temperature with the curve for pure water shown for comparison. The implication of these data is clear from Fig. 8, which shows the relative viscosity (of blood to water) as a function of temperature. Substantial independence of the relative viscosity on temperature is noted above 1 sec.$^{-1}$ of shear rate.

Fig. 9 shows a method of correlation of the yield stress that appears particularly useful. Blood from five different donors shows the same general features (curves 1 to 5). There is a critical hematocrit value H_c below which there is no yield stress whatever. Above this H_c value the cube root of yield stress is linear in the quantity $H - H_c$, where H = actual hematocrit value. H_c values appear to range from 1.3 to 6.5. The yield stress values taken from Fig. 6 are shown on curve 4 of Fig. 9. Curves 1, 2, 3, and 5 were prepared from similar data.

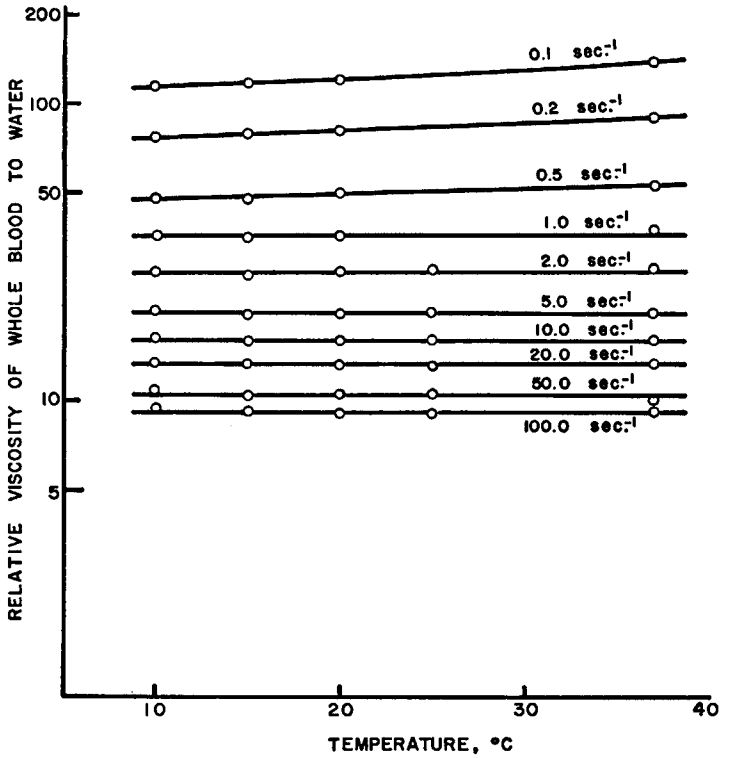

FIGURE 8 Relative viscosity (viscosity of blood/viscosity of water), log scale, *versus* temperature, as computed from Fig. 7.

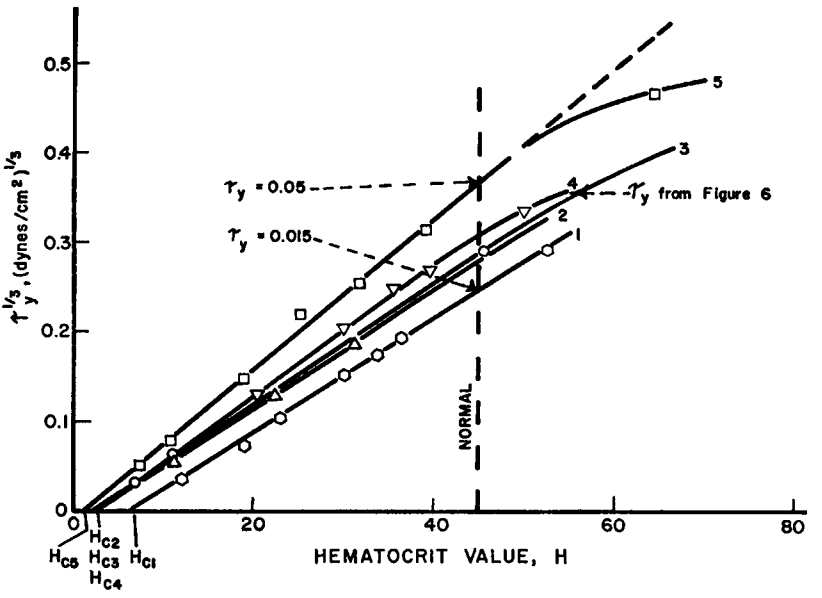

FIGURE 9 Cube root of yield stress *versus* hematocrit value, computed from Fig. 6 and from similar data on other samples of blood.

DISCUSSION OF RESULTS

The Casson equation has been applied to capillary viscometer data on blood, but possibly because of the wall effects, the *extrapolated values* of yield stress are lower by one decade than the values reported here according to one study (8) and higher by three decades according to another (9).

If the physical model on which the Casson equation is based is relevant to blood, then the rod-like aggregates of the model must be rouleaux of red cells. This appears to be consistent with the findings of Fahraeus (10) and the observation of rouleaux formation in blood sheared between microscope slides.

Presumably the temperature independence or near-independence of the yield stress is an indication that energies of interaction holding the red cells together far exceed thermal (Brownian) energy. Above shear rates of 1 sec.$^{-1}$, the constancy of the relative viscosity—its independence of temperature—indicate that the continuous medium (plasma) is the only part of the system affected by temperature. For if at a given shear rate the concentration of aggregates of red cells, or the average of aggregates, or their mode of movement past each other were affected by temperature as well as is the viscosity of the plasma, one could not account for the horizontal curves of Fig. 8.

The approximate linearity of the curves in Fig. 7 (ln η *versus* $1/T°$), and the constancy of the relative viscosity in Fig. 8, from shear rates of 1 to 100 sec.$^{-1}$

combine to give the result that the temperature function of shear dependent viscosity $\eta(\dot{\gamma})$ can be expressed by an Arrhenius type of equation as originally proposed by Andrade, with the *same* activation energy E (cal/mol) as is applicable to *water, viz.*:

$$\eta(\dot{\gamma}) = A(\dot{\gamma})e^{-E/RT}$$

where A $(\dot{\gamma})$ is a function only of shear rate and the nature of the suspension, not of temperature.

R = gas constant
T = absolute temperature

As one examines data progressively close to zero shear rate, (*v.* Fig. 6 near $\dot{\gamma}^{1/2} = 0.1$), the absolute viscosity of blood is becoming both very large in magnitude and also independent of temperature, whereas, of course, the viscosity of water must change with temperature.

This is revealed as the flattened slope of the 0.1 sec.$^{-1}$ curve in Fig. 7 as compared with lower curves. Such a result is to be expected if the dynamic rheology is controlled increasingly by movement of aggregates of rouleaux past each other—in other words by the plastic deformation of three-dimensional networks.

The correlation of yield value with hematocrit value according to the relation

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

appears to be generally valid up to hematocrit levels of 50 with values of A around 0.008. This equation follows, in form, an equation proposed by Norton (11) for clay suspensions. Therein, the volume per cent of dispersed mineral phase corresponded to the hematocrit level as used in the above equation. Bolger (12) in a careful study of dilute kaolin suspensions, which flocculated reversibly into three-dimensional structures, found a close correlation of the yield stress of the suspensions according to the above equation. On the other hand, the data of Bolger could not be even crudely fitted to a Casson plot near zero shear rate, implying that the mechanism of flow in the kaolin suspensions near zero shear rate is different in kind from the flow of red cells in plasma. It therefore is surprising that the yield stress can be correlated with volume per cent suspended matter by the same form of equation. It would appear worth while, from the point of view of gaining biophysical insight into the mechanisms producing yield value, to attempt to find out why the critical hematocrit value H_c varies from person to person to the extent it does, and what factors contribute to the coefficient A which varies by about 20 per cent in the runs shown.

Above hematocrit levels of 50, the yield stress is better correlated by the equation

$$\tau_y = ae^{bH}$$

first proposed by Green (13) for numerous highly concentrated suspensions of pig-

ment in oil. Herein a and b are empirical constants. Although this equation may prove to have clinical utility for predicting yield stress in cases of polycythemia, we suppose that experiments in the more dilute range (5 to 30 hematocrit level) in which the cube root correlation is valid and in which, at the same time, the Casson equation is exactly followed, will finally prove more rewarding.

Haynes and Burton (14), working with suspensions of red cells in saline, found no yield stress whatever, regardless of hematocrit level, in capillary instruments. Since the range of shear rate in capillary instruments is usually high, extrapolation to zero shear rate can be misleading. For example, on page 114 of reference 8, plasma is indicated to have a considerable yield stress, from capillary data, whereas in the range of 0 to 14.92 sec.$^{-1}$ of shear rate,[2] we find all samples of plasma to be Newtonian with absolutely no yield stress detectable.

Gabelnick (15) using an earlier version of the Couette viscometer described herein, noted that, at the same hematocrit level (around 44), suspensions of red cells in saline solution (Ringer's solution) were almost Newtonian in rheology, whereas the same red cells in their native plasma showed highly non-Newtonian rheology at low shear rates. At that time Gabelnick lacked means for accurate torque measurement at zero shear so that he failed to prove the presence of yield stress in plasma suspensions of red cells.

In the light of his experiments, Gabelnick suggested that the plasma proteins must interact with the red cells causing them to associate in some manner, a view certainly concordant with that of Fahraeus (10). Suspensions of red cells in Ringer's solution have been tested in the viscometer described herein and have repeatedly been found to have no yield stress, with dynamic rheology that is virtually Newtonian at hematocrit levels of 44. Consequently, we hope to elucidate in continuing rheological experiments the role of proteins, and for that matter of all other elements present in plasma but not in Ringer's solution, in respect to interactions with the red-cell surface.

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