Viscosity and Clotting of Blood in Venous Thrombosis and Coronary Occlusions

By Leopold Dintenfass, M.Sc., Ph.D., F.R.A.C.I.

In a recent paper on thixotropy of blood and proneness to thrombus formation, Dintenfass stressed four important points. (1) Human blood, normal as well as abnormal, exhibits thixotropy and consequently red cells are aggregated in a reversible manner both in normal and abnormal blood. (2) Viscosity of blood in a few cases of thrombosis and coronary occlusion was found to be up to tenfold greater than that of normal blood. (3) The initial stages of clotting appeared to proceed along different rheological pathways if studied under different rates of shear. (4) It was suggested that excessive thixotropy of blood was an indicator of proneness to thrombus formation.

Some of these concepts are not actually new. Fahraeus envisaged as early as 1921 a possible relationship between aggregation of red cells and thrombus formation. Wasilewski observed that blood was more viscous in cases of cardiac decompensation. Aksyantsev found an increase of blood viscosity in cases exhibiting transient interruptions of cerebral blood circulation. Marin and Stefanini postulated that a sudden coagulation of the blood within an apparently intact vascular channel might result from the combined effects of stasis, or slowing of the blood flow, and of a transient hypercoagulable state. Ziliaci suggested that red cell aggregation might constitute a preliminary stage of thrombosis and that spontaneous thrombosis is preceded by localized or general intravasal aggregation of red cells.

The former and the latter studies differ in that Dintenfass provided a method for a quantitative approach to the problems of aggregation of red cells and thus made it possible to study quantitatively the initial stages of thrombosis. The method is basically simple and requires a determination of viscosity of blood obtained from patients suffering from thrombosis and coronary occlusion is highly significant statistically. In addition, it will be shown that clotting of blood not only proceeds according to the intrinsic properties of the particular blood sample, but also depends on the velocity gradient at which such clotting takes place.

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Methods

ROTATIONAL CONE-IN-CONE VISCOMETER

The cone-in-cone viscometer which measures viscosity of blood over a large range of velocity gradients has been described in an earlier paper. Since that time, however, some improvements have been made; these are shown in figure 1.

The suspension of the torsion wire has been modified to make the strip adjustable in the horizontal plane by means of a micrometer. A precise alignment of two coaxial cones is thus possible using horizontal and vertical micrometers. The old 4-speed gearbox has been replaced by a "Zeromax" stepless drive which may be connected either directly to the rotating cone of the viscometer or, alternatively, through a secondary gearbox giving 150:1 reduction. The torsion strips used are still "Mallory 73" beryllium copper strips of 15 cm length and with cross-sections of 0.001 by 0.025 inch, 0.002 by 0.025 inch, and 0.004 by 0.025 inch.

The Teflon cones employed have angles of 37 and 35 degrees against the vertical axis of the instrument. The angle of the external (rotating) cone is 40 degrees to the axis of the instrument. The instrument was calibrated using standard oils obtained from the National Standards Laboratories (Sydney). The rates of shear are calculated using an equation of Oka the solution of which was given in an earlier paper. The coefficients of proportionality between the rate of shear, \( D \), and revolutions per minute, \( f \), are 1.254, 0.732, and 0.339 for the internal cones of half-angle 37, 35, and 30 degrees, respectively.

BLOOD SAMPLES

Samples of blood were obtained from donors and patients using uncoated needles and syringes. A stop watch was started at the moment of blood removal and the time intervals of subsequent events were noted. The needle was removed from the syringe and blood was poured into the gap between the cones. A sample of one or two milliliters was required. The drive of the viscometer was started and the position of the lightspot was observed on the scale. Speeds were changed without stopping the drive.

Two to four samples of blood were required to determine the viscosity of unclotted blood over the whole range of rates of shear. During the first two to four minutes the viscosity of blood did not change as shown in figures 3 and 4 below. These constant values of viscosity were plotted against the rate of shear on a log-log scale. When clotting of blood was studied, observations of changes of viscosity with time were made on one sample at a constant rate of shear. The results were plotted as the apparent viscosity against the time which had elapsed from the moment of removal of the blood.

Two groups were tested. The first was a control group and consisted of nine males and one female, all members of the departmental staff. Their ages ranged from 19 to 50 years. The second group consisted of patients suffering

<table>
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<tr>
<td>HI†</td>
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<td>WH†</td>
<td>M</td>
<td>54</td>
<td>thrombosis of leg vein</td>
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* Patient from the Prince of Wales Hospital.
† Patient from the Royal Prince Alfred Hospital.
§ Dindevan: phenindione.
** H: hematocrit value expressed as a fraction of the total volume.
†† PI: prothrombin index.
§§ ESR: erythrocyte sedimentation rate.

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FIGURE 1

Modified rotational cone-in-cone viscometer. (1) internal cone; (2) external, rotating cone; (3) thermostatically controlled jacket; (4) torsion strip; (5) micrometer controlling vertical adjustments; (6) micrometer controlling horizontal adjustments; (7) suspension permitting variations in angular inclination of the mirror; (8) locking device; (9) supporting rod marked with circular notches one inch apart; (10) secondary gearbox permitting a 150:1 reduction; (11) primary gearbox "Zeromax"; (12) point of attachment to the tachometer (which is not visible in this picture); (13) mirror; (14) pulley of the secondary gearbox; (15) pulley of the secondary gearbox; (16) pulley of "Zeromax"; (17) large rubber corks which serve as the anti-vibration mounting; (18) ballast (10 lb of lead) required to increase stability of the viscometer; (19) screws permitting a horizontal adjustment of the base of the viscometer. A range of low rates of shear is obtained when pulleys are interconnected as illustrated. A range of high rates of shear is obtained if pulley (16) of "Zeromax" is directly connected with the rotating cone (2).
from venous thrombosis, coronary occlusion, and from polycythemia.

To identify results the members of the control group are designated by letters as follows:—AC, male, age 55; AN, male, age 22; DA, male, age 19; DO, male, age 50; DD, male, age 50; FE, male, age 45; GL, female, age 23; JE, male, age 45; RO, male, age 20; WE, male, age 32. Hematocrits of the control subjects were between .38 and .43. The coding of blood samples obtained from patients is contained in table I.

It is realized that the control and the thrombosis groups are not well matched for age or hematocrit. For comparison blood samples from three sheep were also studied.

**Results**

Experimental results were plotted either in the form of viscosity-time plots on a numerical scale, or in the form of viscosity-rate of shear plots on a log-log scale. Because the terms "rate of shear" and "velocity gradient" are used continuously in this study, and because these terms are not common ones, an explanation is in order. The "rate of shear," or the "velocity gradient," is a function of the velocity of fluid laminae or of the surfaces surrounding the fluid, and the geometry of these surfaces. In the simplest case of two parallel surfaces, spaced apart by a gap d, and moving with relative velocity V, the velocity gradient between these two surfaces is equal to V / d; units of velocity, cm/sec, divided by the units of distance, cm, give units of rate of shear, sec⁻¹.

It was decided to restrict studies to blood in the absence of anticoagulants because it was found in a series of cases, two of which are illustrated by figure 2, that addition of anticoagulant in vitro results either in an increase or in a decrease of the blood viscosity, depending on the type of anticoagulant used. Thus, it became apparent again that in order to obtain meaningful results one should not add anticoagulants in vitro. Repeated testing of blood samples obtained from the two patients held on Dindevan (phenindione) showed that results were not reproducible. Consequently, all viscosity studies were done on fresh blood in the absence of anticoagulants. However, determinations made in the absence of anticoagulants introduced the possibility that some changes in blood viscosity take place immediately after blood is removed from the donor's vein. A large series of blood samples were studied by viscometric methods from the moment of extraction until the onset of clotting. As shown in figures 3, 4, and 5, the viscosity of blood remains constant over a period of two to six minutes, depending on the sample and on the method of testing.

It was surprising to find that clotting was consistently more rapid when higher rates of shear were used. The latent period before the onset of clotting at low rates of shear may be
Clotting of blood in control group of healthy subjects. Viscosity of blood, in poises, is plotted against time, in minutes, which had elapsed from the moment of removal of blood from the vein. Rates of shear at which clotting was followed are indicated on the curves. Experimental curves correspond to the blood of the following donors: 1: WE, 2: FE, 3: RO, 4: DD, 5: AC, 6: DA, 7: GL, 8: DO, 9: JE. Tests were done at 36°C.

as much as twice as long as the respective latent period at high rates of shear (experiments 6 and 9, figure 3; experiments 3, 4, 6, and 12, figure 4). It seems clear that the rate of clotting of any single blood sample depends on the velocity gradient at which such clotting takes place. Such behavior is not restricted to human blood but applies also to blood of sheep (fig. 5). When, however, clotting is followed not at normal blood temperature, but at room temperature (20° to 25°C), this characteristic pattern is reversed, i.e., clotting takes place more rapidly at lower rates of shear.

In studying viscosity of whole blood it was necessary, as the first step, to establish variations in the viscosity of blood obtained from voluntary donors, all of whom were known to be in good health. The spread of data (fig. 6) is due partially to variability of the hematocrit value and partially to natural differences in the degree of aggregation of red cells. Viscosity studies done on blood samples from a series of patients suffering from coronary occlusion, venous thrombosis, and polycythemia are illustrated by figure 7. The range of viscosities observed, at the rates of shear corresponding to those used for the control group, shows clearly a pronounced increase. Two types of statistical treatment
have been adopted. The first one, the distribution free significance test, indicated on "null hypothesis" that the probability of ten paired samples showing the same sign of difference in viscosity is equal to \((1/2)^{10} = 1/1024\). The second test was a parametric test based on log distribution using the \(t\)-test. The comparison was carried out in the thixotropic region at the rate of shear of 0.1 sec\(^{-1}\). The following data, including degrees of freedom, \(n\), arithmetic means, \(\bar{x}\), and standard deviations, \(s\), were calculated for the two groups:—In group A, healthy persons: \(n_a = 9\); \(\bar{x}_a = 0.446\); \(s_a = 0.188\); \(n_b = 0.198\). In group B, patients: \(n_b = 11\); \(\bar{x}_b = 1.12\); \(s_b = 0.220\); \(s_b = 0.230\). These results were tested to see whether the two groups differed significantly in their means, or whether they should be regarded as belonging to the same population. The following equations were employed (from Fisher, *Statistical Methods for Research Workers*, 1956):

\[
s^2 = \frac{1}{n_a + n_b} \left[ \frac{\sum (x_a - \bar{x}_a)^2 + \sum (x_b - \bar{x}_b)^2}{n_a + n_b - 2} \right]
\]

\[
t = \frac{\bar{x}_a - \bar{x}_b}{s} \sqrt{\frac{(n_a + 1)(n_b + 1)}{n_a + n_b + 2}}
\]

The computed data gave the following values:

\(\bar{x}_a - \bar{x}_b = 0.674\) (where \(0.674 = \log \frac{100}{80}\)).

**FIGURE 4**

Clotting of blood in the group of patients suffering from venous thrombosis (graphs 4 and 11), coronary occlusion (graphs 1, 2, 5, 6, 7, 9, and 10), and polycythemia (graphs 3 and 8). Viscosity of blood, in poises, is plotted against time, in minutes, which had elapsed from the moment of removal of blood from the vein. Rates of shear at which the clotting was followed are indicated on the curves. Experimental curves correspond to the blood of the following patients: 1: LO, 2: RO, 3: RI, 4: WI, 5: BU, 6: JWD, 7: BR, 8: TR, 9: MA, 10: BR, 11: RI, 12: LU. Tests were done at 36° C.
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Clotting of blood samples from three sheep. Viscosity of blood, in poises, is plotted against time, in minutes, elapsed from the moment of the removal of blood. Rates of shear at which the clotting was followed are indicated on the curves. Curve 1: sheep no. 131, 2: sheep no. 39, and 3: sheep no. 897. Viscosity determinations were done at 36°C in experiments 1 and 2, and at 25°C in experiment 3.

The third section of results considers aggregation of red cells. A quantitative method, developed by Dintenfass for determining the degree of aggregation of colloidal particles in suspensions, was adapted here for assessment of the aggregation of red cells. The modified diagram (fig. 8) relates the degree of aggregation (flocculation), $\beta$, the viscosity ratio, $\eta/\eta_s$, the sedimentation volume at rest, $F$, and the hematocrit, $F_x$, expressed as volume fractions instead of as per cent of the volume.

The degree of aggregation of red cells can be assessed from the diagram (fig. 8) if the hematocrit and the flow curve of a blood sample are known. This method is approximate only, because some degree of error is introduced by slight variations in the viscosity of plasma and in the viscosity of the red cell interior as a function of velocity gradient.

A series of sedimentation tests was done in order to test this diagram. Sedimentation volumes in Westergren tubes, after 24 hours, were taken as the sedimentation volumes at rest. The experimental and the calculated re-
Results differed (± 20%), the sign of this difference depending on the type of anticoagulant used in the sedimentation test. It was observed not only that the various anticoagulants exerted a different effect on sedimentation volume of a particular sample of blood, but also that different samples of blood responded in an unpredictable manner if treated with the same anticoagulant. This problem needs further study.

The computed data are contained in Table 2. While the diagram (Fig. 8) is not assumed to be final, it supplies a way for estimating the degree of aggregation of red cells and/or sedimentation volumes in the absence of anticoagulants.

A purely qualitative indication of the aggregation of red cells may be obtained directly from the viscometric data. The ratios of viscosities determined at high and at low rates of shear are compared for the blood obtained from healthy subjects and from patients suf-

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**Figure 5**

Flow curves of whole blood of patients suffering from venous thrombosis (experimental points 3, 9, 10), coronary occlusion (experimental points 1, 2, 4, 5, 6, 7, 8, 11), and polycythemia (experimental points 12). The viscosity of blood was tested by means of a cone-in-cone viscometer, at 36°C, and in the absence of anticoagulants. Abscissa: viscosity, in poises; ordinate: the rate of shear in reciprocal seconds on a log-log scale. The experimental points correspond to blood viscosity of the following patients: 1: MA, 2: GL, 3: RI, 4: MT, 5: BR, 6: JWD, 7: BO, 8: LO, 9: LU, 10: WI, 11: BW, and 12: RJ.

It may be observed that a tenfold difference can exist between the viscosity ratios of the two series of blood samples.

The fourth section of results deals with the thixotropic recovery and the time-dependence of blood viscosity. In order to resolve the conflict between the advocates of "shear thinning" (which is only a function of the rate of shear) and the advocates of "thixotropy" (which is a function of both the time and the rate of shear), the viscosity of blood samples was tested in the following way. The viscosity was measured, first, in the usual way, from low rates of shear towards high rates of shear, and then back at decreasing rates of shear. Both sets of experimental data were expected to supply the same answer if time-dependent phenomena were absent. However, these data differed, the second set of data be-
TABLE 2

Degree of Aggregation of Red Cells Estimated by Means of Diagram Illustrated by Figure 8

<table>
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</tr>
<tr>
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<td>LU</td>
</tr>
<tr>
<td>WE</td>
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<td>BW</td>
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</table>

Note that the degree of aggregation, $\beta$, is described, by definition, as a ratio of the sedimentation volume at rest to the hematocrit value.

TABLE 3

Qualitative Indication of the Aggregation of Red Cells by Means of the Viscosity Ratio, $\eta_{0.4}/\eta_{100}$

<table>
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Note that the difference in viscosity ratios, between healthy subjects and patients suffering from venous thrombosis and coronary occlusion, would greatly increase if the low-rate-of-shear viscosity were determined at rates of shear lower than 0.4 sec$^{-1}$.

$\eta_{0.4}$ = viscosity determined at 0.4 sec$^{-1}$.

$\eta_{100}$ = viscosity determined at 100 sec$^{-1}$.

plotted also on a log-log scale (fig. 9). A more advanced treatment of thixotropic re-

FIGURE 8

A modified diagram (after Dintenfass 14) permitting an assessment of the degree of aggregation of red cells. This diagram gives relationships between the sedimentation volume at rest, $F$, the sedimentation volume at total dispersion (which is equal to hematocrit), $F_*$, the degree of aggregation, $\beta (=F/F_*)$, and the viscosity ratio, $\eta/\eta_*$; where $\eta$ is the viscosity of suspension at 0.4 sec$^{-1}$, and $\eta_*$ is the viscosity of suspension at 80 to 100 sec$^{-1}$. Note that $F$ in this diagram is expressed in per cent of the total volume. This diagram permits an approximate estimation of $\beta$ and $F$, if $F_*$ and $\eta/\eta_*$ are known; or vice versa.

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<table>
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<td></td>
<td>90</td>
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Note: this blood sample was sheared at the rate of 1.12 sec⁻¹, while its thixotropic recovery was followed at 0.046 sec⁻¹.

Thixotropic recovery could be carried out according to Dintenfass.

Discussion

VISCOSITY AND FLOW TYPES OF NORMAL AND ABNORMAL BLOOD

Viscosity of blood obtained from patients suffering from venous thrombosis and coronary occlusion was found to be much greater than viscosity of blood from healthy donors. This is especially true if blood samples are compared at very low rates of shear. At high rates of shear, on the other hand, the structural viscosity disappears and the blood viscosity becomes directly related to the hematocrit.

Changes in structural viscosity are due to variability of the reversible aggregation of red cells. The degree of aggregation increases when the velocity gradient decreases and, vice versa, it decreases when the velocity gradient increases. A complete disaggregation (deflocculation) takes places at some critical rate of shear which is about 6 sec⁻¹ for normal blood, and up to 60 sec⁻¹ for abnormal blood.

There is a tendency to describe the rheological type of blood flow as “shear thinning.” It should be stated emphatically that blood is thixotropic, i.e., its viscosity is affected both by time and rate of shear. The time-dependence of blood viscosity, while not readily apparent at higher rates of shear, is quite obvious if studied at rates of shear about 0.01 sec⁻¹. A rapid shearing of blood at high rates of shear, followed by a determination of viscosity at low rates of shear, shows that the thixotropic recovery time is not insignificant and, indeed, it may require a period of 20 seconds to 2 minutes (table 4, fig. 9).

This statement does not contradict the evidence presented in the literature even if it appears to do so at first sight. It has been shown already by Dintenfass that the thixotropic recovery time depends on the rates of shear employed during the experiment. Dintenfass stated that “the thixotropic recovery between (colloidal and molecular) equilibrium states corresponding to higher rates of shear is very rapid by comparison with the recovery between equilibrium states corresponding to some lower rates of shear.” As other investigators employed high rates of shear in their studies of blood, it should not be astonishing that they were not able to notice these time-dependent phenomena.

![FIGURE 9](http://circres.ahajournals.org/)

Thixotropic recovery of a normal blood sample (WE). Sample was sheared at 1.12 sec⁻¹ and the recovery followed at the rate of shear of 0.046 sec⁻¹. Data (as in table 4) are plotted as the recovered viscosity in poises, \( \tau^* \), against the thixotropic recovery time in seconds, \( t \), on a log-log scale. The extrapolated curve permits an approximation of thixotropic recovery times between levels of equilibrium rates of shear higher or lower than the ones employed in this experiment. Note that the thixotropic recovery times are extremely short at high rates of shear (lower part of graph) but quite long at low rates of shear (top part of graph). A more exact curve of thixotropic recovery times could be plotted according to reference 15, figures 9 and 10.

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The degree of thixotropy will be much more pronounced in the systems showing highest viscosities at low rates of shear. Consequently, these phenomena are easier to observe on the blood from patients than on the blood from donors, and they might be more difficult to observe on the blood samples containing anticoagulants.

According to statistical analysis, normal blood samples and abnormal blood samples present two distinct population distributions (where the term “population” is used in its statistical meaning). The viscosity of abnormal blood, i.e., blood from patients with thrombosis and coronary occlusion, may be tenfold higher than that of the normal blood. While the significance of this should not be overemphasized, its relevance to cardiovascular problems will be reviewed in a later section of this discussion.

CLOTTING OF BLOOD

A study of blood coagulation in vitro by means of a cone-in-cone viscometer indicates that the rate of clotting depends on the velocity gradient. In most samples the time of clotting was much shorter at high velocity gradients than at low velocity gradients, if the tests were carried out at 36 to 37°C. It is difficult, at this stage, to supply an explanation for this phenomenon. It was observed, however, that the above pattern is reversed when the clotting is followed at room temperature, instead of at blood temperature. When the temperature of clotting in vitro is reduced to about 20°C, the apparent clotting takes place more rapidly at very low rates of shear. In other words, when the chemical reaction of plasma clotting is delayed by a decrease in temperature, a pronounced aggregation of red cells appears to take place in the time period during which the plasma clotting itself is not yet evident. One can presume that some process, as yet unknown, goes on during the initial stages of clotting, this process involving a rapid aggregation of red cells.

This concept is neither new nor unusual. Grégoire found that blood coagulation in insects proceeds mainly through cellular clotting. Heilbrunn stated that it should be recognized that a clot can be formed by an aggregation of blood cells alone. While many authors might consider that clotting does not occur if a formation of fibrin or fibrin-like substance does not take place, Copley, among few, appreciated the importance of cellular aggregation. It should be emphasized that the red cell itself may contain and extrude clotting initiators.

Aggregation of red cells does not actually require specific agglutinins; it requires that the adhesion forces (i.e., London, Van der Waals, electrostatic charge, etc.) between surfaces of the individual red cells be higher than the adhesion forces between an individual red cell and the surrounding plasma. The forces of adhesion will be influenced by the presence of various polar-nonpolar compounds showing surface-active properties.

AGGREGATION OF RED CELLS AND ITS CAUSES

It is pertinent to consider some of the causes for aggregation of red cells. The aggregation of red cells has to depend inherently on their surface characteristics and on the effect of surface-active macromolecules adsorbed onto the red cell surface. It is already known, for instance, that an excessive level of fibrinogen in blood causes red cells to sediment rapidly. A high globulin level may be responsible for an accelerated sedimentation and a correspondingly increased degree of aggregation of red cells. An infection by viruses may lead to aggregation or agglutination of red cells. The aggregation of red cells was ascribed by some to the electrical surface potential. Rigid aggregates were observed by Madow and Bloch in cases of pneumococic pneumonia, acute myocardial infarction, malaria, etc. While the present author agrees with Madow and Bloch and Knisely that a pronounced but intermittent sedimentation of red cells takes place in vivo, such sedimentation being a direct consequence of the reversible aggregation of red cells (as proved by the presence of thixotropy), he does not agree that such a sedimentation is restricted only to pathologic
BLOOD VISCOSITY IN THROMBOSIS

cases. The presence of thixotropy in the blood of healthy subjects indicates that a reversible sedimentation must take place even during the life of such healthy persons.

THIXOTROPY OF BLOOD AND PRONENESS TO THROMBOSIS; RHEOLOGICAL CONSIDERATIONS

Detection of impending thrombosis and initial atherosclerosis is one of the unsolved problems in medicine. In many cases diagnosis is made post mortem because of lack of methods for the detection and assessment of pathologic states. However, it was suggested recently that one of the factors involved in these conditions is a change in the fluid structure as determined by blood rheology. An intuitive and empirical appreciation that the physical properties of blood might be a cause, or an effect, of various diseases has been expressed repeatedly from the fourth century B.C. to modern times. Experiments with bloodletting and observations on the "buffy coat" might be considered forerunners of what is now described as hemorheology.

Thrombosis and atherosclerosis are known to affect various sites of the circulatory system. Consequently, if the viscosity of blood is to account, at least partially, for these phenomena, it should be capable of exhibiting various values at different sites of the circulatory system; i.e., the rheological parameters should show pronounced variation at these different sites. This is indeed the case. Blood is a thixotropic fluid and, thus, its viscosity shows both time- and rate-of-shear-dependence. Blood is a multiphase fluid and its viscosity is influenced by three dynamic and interdependent mechanisms. (1) The flocculation-deflocculation equilibrium of red cells will be shifted according to the values of the velocity gradient. (2) The velocity gradient will affect the sol-gel-sol transformations of the red cell interior. (3) The molecular and colloidal structure of the plasma will undergo a change, influencing viscosity of plasma itself and viscosity of whole blood (fig. 10).

This triple thixotropic mechanism, each of whose components shows a different response to time and rate of shear, is superimposed on the intrinsic properties of flow in circular vessels in which the profile of velocity gradient requires a zero rate of shear in the axis of the vessel. This profile must be also a function of the pulse, of the pressure gradient, of the visco-elasticity of vessels walls, and
of the smoothness of the endothelial lining. Consequently, great variability of thixotropy, aggregation of red cells, viscosity of plasma and blood, can exist in various portions of the circulation. Flocculation (aggregation) of red cells will always take place when the flow rate is below a certain critical value. The larger the aggregates, the greater is their tendency for sedimentation. The latter is counteracted by axial flow and by the thixotropic properties of plasma. Setting out from the axial stream, where plasma viscosity and whole blood viscosity are highest, is much slower than settling from the peripheral regions. When, however, the aggregates reach a certain size, an intermittent sedimentation should take place. It should be mentioned also that aggregates are present in a distribution of many sizes. Aggregates may be expected to be most prevalent in larger veins and arteries, irrespective of the nominal rate of shear at the vessels wall, as long as laminar conditions prevail. Sedimentation is accentuated by eddies or by changes in the velocity of the main stream.

Hydrodynamic and rheologic factors are not a matter of conjecture. It is also clear from statistical analysis of the data presented, that a significant difference exists between the viscosity (and, thus, aggregation) of blood from healthy subjects, on the one hand, and the viscosity of blood from patients suffering from venous thrombosis and coronary occlusion, on the other hand. Excessive aggregation of red cells, and thus excessive thixotropy, are related to thrombosis, but the question of their significance as a contributing cause or a sign of thrombosis, remains to be answered. The following inference can be drawn. It has been observed that cells pressed together in an aggregate or sludge disintegrate and are ingested by phagocytes. Ponder suggested that breaking up of aggregates or sludges carried in a stream of blood in vivo, if accompanied by even partial lysis, would represent considerable hemolysis because so many sludges form and break in the course of the lifetime of an individual red cell. This process is significant because it is usually assumed that an activation of clotting can take place only when blood is exposed to a foreign surface. However, it was observed that localized stasis of blood leads to the production of serum prothrombin conversion accelerators. These in turn, just as a foreign body would do, can trigger the mechanism of intravascular thrombosis.

Direct proof linking viscosity, thixotropy and aggregation of red cells in blood tested in vitro, on the one hand, and the proneness to thrombosis, on the other hand, in a causative way requires a follow-up study of a random group of persons over many years. While the present author recommends such method, he is in no position to carry it out. An alternative approach, used in this investigation, is based on the assumption that persons showing a history of thrombosis should show also an excessive thixotropy of blood. In addition, the role of platelets is not forgotten. A current study (Rozenberg and Dintenfass, to be published) indicates that the behavior of platelets depends also on the velocity gradient. It is suggested that students of thrombo-embolic diseases should recognize two new parameters of coagulation: 1. an effect of the velocity gradient, and 2. an effect of the aggregation of red cells, the latter being identified by measuring the thixotropy of blood.

Summary

Viscosity of blood was measured in the absence of anticoagulants by means of an improved cone-in-cone viscometer. Data obtained in a study of a series of patients suffering from venous thrombosis and coronary occlusion were compared with data from a control group of healthy subjects. The probability that the large difference in viscosity observed between normal and abnormal blood is due to chance is approximately 1 in 1,000,000. The viscosity of blood remains constant, at any rate of shear, during the first two to four minutes after removal of blood. Clotting starts after various time periods, depending not only on the intrinsic properties of the particular blood sample, but also on the velocity gradient at which such clotting takes place.
The following general trends were established: at 36°C the onset of clotting is much more rapid at high rates of shear than at low rates of shear. It appears, however, that at lowered temperatures this trend is reversed.

The causes of aggregation of red cells, and the role of this aggregation in clotting, are discussed. It is emphasized that blood is thixotropic, i.e., that its viscosity is both time and rate-of-shear dependent. Thixotropy of blood is due, mainly, to a reversible aggregation of red cells. This suggests the existence of intermittent sedimentation of red cell aggregates during flow in blood vessels. Owing to the existence of a velocity profile, in which the axial region is under zero or near-zero rate of shear, such aggregates should be present especially in large veins and arteries. It appears that an increased thixotropy and viscosity are symptomatic of some thrombotic states.

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