Effect of Velocity Gradient on the Clotting Time of Blood and on the Consistency of Clots Formed in Vitro

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

Recent studies from this laboratory have shown that the clotting time of blood and the morphology of blood clots can be altered markedly by flow of blood during the period of clotting. The rheology of clots has been studied by Hartert, de Nicola, and Marchal et al. by means of thrombelastography, while Scott Blair and Copley and Scott Blair employed a gelometer. However, no observations have been published on the functional relationship between the consistency of the blood clot and the rates of shear at which such clot (or coagulum) is formed.

In the present paper I will show (a) that there exists a quantitative relation between clotting time of blood and the velocity gradient, and (b) that the consistency of clots (or blood coagula, or artificial thrombi) is directly dependent on the velocity gradient at which such clots are cast. In addition, the manner in which rheological techniques may indicate the molecular and colloidal structure of clots will be discussed.

Methods

INSTRUMENTATION

Two instruments were used for the present study. The first was a cone-in-cone rotational viscometer, equipped with a ring-in-ring adapter. The rotational viscometer permits measurement of viscosity of blood over a large range of rates of shear; it is also a sensitive tool for following changes in viscosity during the initial stages of coagulation. The study was done at 37°C, the temperature of the viscometer being controlled by a mercury-contact thermometer and relay. Deflections of the internal cone or ring, suspended on copper-beryllium torsion strips, were observed as movements of a lightspot on a semicircular plastic scale.

The second instrument was constructed specifically for this study. This is a variable-frequency oscillating viscometer of fixed amplitude of 140 degrees. The instrument (named "variable-frequency thromboviscometer," VFTV) allows observation of changes in the viscosity of coagulating blood. Plots of experimental data result in tuning fork-like curves; these are similar to the records obtained from the Hartert thrombelastograph. VFTV has a cone-in-cone geometry, the torsion strip being a copper-beryllium strip, 15 cm long and 0.075 x 0.008 inches in cross section.

The coefficient of proportionality between the rate of shear, D, and the number of revolutions per minute, f, equals 0.732 for the cone-in-cone geometry, and 2.1 for the ring-in-ring geometry. The mean rates of shear for VFTV have been calculated as follows:

Mean rate of shear = frequency x (140°/360°) x 0.732.

In contradistinction to the rotational viscometer, the rates of shear in VFTV are not constant in time; i.e., the rate of shear fluctuates within the period of each oscillation. The amplitude of...
deflections of the internal cone, which reflects the viscosity of the blood coagulum, also affects the accuracy of the rate of shear. When amplitude of the internal cone is 30 degrees, the rate of shear will be decreased by 21% of the rate of shear calculated for the stationary internal cone. This means that in the final stage of coagulation, when the actual coagulum is already formed, the rates of shear are decreased by 21%. When the deflections of the internal cone are less than 30 degrees, effects on the rates of shear are proportionally less.

The semicircular scale is divided in arbitrary units (1 cm length), each of which corresponds to 2.15 degrees of deflection.

**BLOOD SAMPLES**

Samples of blood were obtained from donors and patients by means of uncoated needles and syringes. A stop watch was started at the moment of blood removal and the time intervals of subsequent events were noted. One milliliter of blood was required for each test. At least two tests were done on each blood, but in a number of cases donors agreed to three consecutive venepunctures.

The total population of donors included 42 healthy normal subjects, 43 patients suffering from myocardial infarction or peripheral arterial disease, 6 patients having polycythaemia, 6 patients with Waldenström macroglobulinaemia, and a group of 12 patients with various diseases (including venous thrombosis, cancer and anaemia). These donors happened to be available, and no conclusions will be drawn, at this stage, with respect to results of clotting tests in various diseases. Reproducibility of experimental techniques was established by consecutive tests, the results of which were within ±10% for clotting times, and ±5% for viscosity data.

**CLOTTING TIMES**

The clotting time of blood, as tested by the instruments described, is the time elapsed from the moment when the first drop of blood appears in the syringe during venepuncture, to the moment when blood viscosity starts to increase due to the coagulation process.

**RHIOLOGIC NOMENCLATURE**

The "rate of shear," or the "velocity gradient," is a function of the relative velocity of the fluid laminae or of the surfaces surrounding the fluid, and of the geometry of these surfaces. In the simplest case of two parallel surfaces spaced apart by a gap d, and moving at a relative velocity V, the velocity gradient between these two surfaces is equal to V/d; the units of velocity (cm/sec) are divided by the units of distance (cm) in order to obtain the units of the rate of shear (sec⁻¹).

The term "thixotropic" is used to describe a system in which viscosity depends on the time and the rate of shear, decreasing as the rate of shear increases. A thixotropic system is reversible, the thixotropic recovery time ranging from a microsecond to a number of hours, depending on the system and on the levels of rates of shear between which it is determined.

**Results**

**CLOTTING TIME TESTS**

Clotting times were determined mainly by means of the cone-in-cone viscometer, the viscosity changes due to coagulation being followed at certain constant rates of shear. Usually, the rates of shear employed were either in the range 0.3 to 0.9 sec⁻¹, or in an intermediate range of 59 to 72 sec⁻¹. Some tests were done in a range of 400 to 600 sec⁻¹, a ring-in-ring adapter always being used in such tests.

**TABLE 1**

<table>
<thead>
<tr>
<th>Group</th>
<th>n†</th>
<th>x</th>
<th>x − s</th>
<th>x + s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clotting times determined at rates of shear over 1 sec⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A 2</td>
<td>42</td>
<td>2:42</td>
<td>2:8</td>
<td>3:28</td>
</tr>
<tr>
<td>B 2</td>
<td>42</td>
<td>3:15</td>
<td>2:32</td>
<td>4:10</td>
</tr>
<tr>
<td>C 6</td>
<td>4:32</td>
<td>3:10</td>
<td>6:29</td>
<td></td>
</tr>
<tr>
<td>D 6</td>
<td>5:46</td>
<td>3:10</td>
<td>10:30</td>
<td></td>
</tr>
</tbody>
</table>

Clotting times determined at rates of shear 59 - 72 sec⁻¹

<table>
<thead>
<tr>
<th>Group</th>
<th>n†</th>
<th>x</th>
<th>x − s</th>
<th>x + s</th>
</tr>
</thead>
<tbody>
<tr>
<td>A 41</td>
<td>2:22</td>
<td>1.58</td>
<td>2:53</td>
<td></td>
</tr>
<tr>
<td>B 43</td>
<td>2:22</td>
<td>1.56</td>
<td>2:55</td>
<td></td>
</tr>
<tr>
<td>C 6</td>
<td>3:26</td>
<td>2.51</td>
<td>4:33</td>
<td></td>
</tr>
<tr>
<td>D 6</td>
<td>4:10</td>
<td>2.53</td>
<td>6:0</td>
<td></td>
</tr>
</tbody>
</table>

*All tests done at 37°C, by means of the cone-in-cone rotational viscometer.
†: number of tests; x: arithmetic mean; s: standard deviation.

Note that all statistical calculations were based on log-normal distribution of clotting times. Numerical values inserted in the above table were obtained from logarithms.

A: healthy donors; B: patients suffering from cardiovascular diseases (myocardial infarction and arterial thrombosis); C: patients suffering from polycythaemia; D: patients suffering from macroglobulinaemia.

*Circulation Research, Vol. XVIII, April 1966*
In confirmation of earlier results\textsuperscript{1,2} it was found that the viscosity of freshly shed blood does not change during the initial stage of the coagulation process. The duration of this initial period, or "rheologically-latent period," varied from a minute to a few minutes. The clotting time was found to decrease as a function of the rate of shear employed during the coagulation process (fig. 1, table 1). At rates of shear about and above 400 sec\textsuperscript{-1} the viscosity increase became apparent immediately after the viscometer started to operate.

It must be emphasized that all the data on clotting time of blood include the first 60 seconds taken by the preparatory work, i.e., by the venepuncture and filling of the viscometer. The rates of shear in a syringe might vary greatly, but are estimated to be in the intermediate range of rates of shear. It is likely that the preparatory period influences the final clotting time of blood, the clotting times observed at low rates of shear being shortened, while the clotting times observed at high rates of shear are prolonged. If it were valid to compensate for the preparatory period by estimating certain corrections in the

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig1.png}
\caption{Clotting times of blood as a function of the velocity gradient. Mean values of clotting times of human blood, obtained from 100 donors, are plotted against $\sqrt{D}$, $D$ being the rate of shear. For clarity, a second scale is provided on which numerical values of rate of shear are marked. Broken horizontal line indicates the beginning of tests; the first 60 seconds being taken by preparatory work. Areas described as a, b, and c represent schematically the frequency distribution of clotting times at three ranges of rates of shear; the coordinate of rate of shear should be read as an arbitrary scale of frequency. Function of rate of shear, employed in this figure, was selected in order to obtain a straight line. It is interesting that aggregation of platelets was found also to be proportional to the same function of the rate of shear.}
\end{figure}
clotting times, the relationship obtained might be as indicated by the dotted line of figure 1. However, not only the clotting time is affected by the velocity gradient, but the consistency of clots is also altered.

b. CONSISTENCY OF BLOOD COAGULA

Blood coagulation was followed also by means of the variable-frequency thromboviscometer (VFTV), freshly shed blood being used. During the first latent period, when the blood viscosity remains constant, oscillations of the outer cone are not transmitted to the suspended inner cone; the torsion strip, the inner cone, and the attached mirror remain stationary. However, as the viscosity of blood increases, a certain amount of movement is transmitted to the suspended inner cone. Typical graphs, plotted from experimental data on coagulating blood, are shown in figure 2.

The significance of decreased amplitude of deflection can be understood best by reference to figure 3. The data of figure 3 were obtained on a Newtonian fluid (a medicinal paraffin oil) which was tested over a range of frequencies. The amplitude of the deflections is proportional to frequency of oscillation. With blood coagula, on the other hand, the amplitude of deflections decreases with increasing frequencies of oscillation; i.e., the viscosity of blood coagula decreases as the frequency of oscillation increases.

The graphs describing consistency of coagula have the shape of the oscillations of a tuning fork, and resemble thus the records commonly obtained by means of thrombelastography.\(^5\)\(^7\) Here, however, both the amplitude and the clotting time are a function of the frequency of oscillation or, in other words, a function of the velocity gradient. As the instrument is calibrated in terms of viscosity, the amplitude observed can be converted into poises (table 2).

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CLOTTING TIME AND CONSISTENCY OF BLOOD CLOTS

It should be noted that the viscosity of blood (freshly drawn and not coagulated), from which the clot is cast, depends also on the rate of shear. Due to the known dependence of blood viscosity on the rate of shear, the lower the rate of shear that is used during coagulation, the higher the viscosity of the original blood. When clots are cast at rates of shear near 1 sec⁻¹, the clot structure evolves from blood having a viscosity ranging from 0.7 to 2 poises. When, however, blood coagulates at intermediate rates of shear, 40 to 70 sec⁻¹, this blood, prior to the increased viscosity induced by coagulation, has a viscosity of only 0.04 to 0.10 poise. Consequently, we can observe a 100- to 230-fold increase of viscosity during coagulation that proceeds at low rates of shear, but only a 30-fold increase of viscosity during coagulation that

### TABLE 2
Increase of Blood Viscosity During Coagulation Proceeding at Different Frequencies of Oscillation in Variable-Frequency Thromboviscometer.

<table>
<thead>
<tr>
<th>Frequency cycles/min</th>
<th>Approximate rate of shear sec⁻¹</th>
<th>Viscosity at 37°C Fresh blood poise</th>
<th>Viscosity at 37°C Clot poises</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.6</td>
<td>1.8</td>
<td>0.08</td>
<td>125</td>
</tr>
<tr>
<td>33</td>
<td>9.4</td>
<td>0.2</td>
<td>20</td>
</tr>
<tr>
<td>166</td>
<td>44</td>
<td>0.08</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>400*</td>
<td>0.04</td>
<td>0.04</td>
</tr>
</tbody>
</table>

* Determination by rotational viscometers (ring-in-ring).

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**FIGURE 4**
Consistency of blood coagula cast at different frequencies of oscillation. Rheology of blood coagula formed in the variable-frequency thromboviscometer was studied on samples of blood obtained from the same donor. Blood was permitted to coagulate at 37°C, at frequencies of 6.6 cycles per minute, 66 cycles per minute, and 166 cycles per minute. Casting frequencies are marked by double circles. Curve A was obtained after the blood sample coagulated for a period of 20 minutes, the frequency employed during coagulation being 6.6 cycles per minute. Curve B was obtained after the blood sample coagulated for 10 minutes the frequency employed during coagulation being 66 cycles per minute. Curve C was obtained after the blood sample coagulated for a period of 8 minutes, the frequency employed during coagulation being 166 cycles per minute. Curves are plotted as viscosity \( \eta \), against the frequency, \( F \), or rates of shear, \( D \).

*Source: Circulation Research, Vol. XVIII, April 1966*
proceeds at intermediate rates of shear. There is virtually no increase in viscosity of coagulating blood when coagulation takes place at rates of shear above 400 sec\(^{-1}\) (table 2).

c. THIXOTROPY OF BLOOD COAGULA FORMED IN VITRO

The following experiments were done to investigate the viscosity of coagula as a function of rate of shear and to study the thixotropic properties of coagula. A series of blood samples was permitted to coagulate at oscillation frequencies of 6.6, 33, and 166 cycles per minute. After their formation, these coagula were tested over the whole available range of frequencies (fig. 4). The "casting" frequency is denoted by a double circle. It is evident that the coagulum cast at a frequency of 6.6 cycles per minute (curve A) shows the highest viscosity over the whole range of scanning frequencies. The viscosity of this coagulum decreased as the testing frequency increased, that is, as the rate of shear increased.

The coagulum cast at a frequency of 66 cycles per minute (curve B) shows relatively lower viscosity over the whole range of frequencies. It is interesting to note that the viscosity of this coagulum increases when the frequency (and thus the rate of shear) decreases. When, however, this coagulum was deformed for a few minutes at a frequency higher than that of the casting, a pronounced decrease in the viscosity took place over the whole range of available frequencies. The blood coagulum formed at casting frequency of 166 cycles per minute exhibits the lowest over-all viscosity; in this case viscosity increased slightly when the testing frequency decreased.

The above pattern of behaviour, in which viscosity increases when the rate of shear decreases, and vice versa, is typical of thixotropic fluid. The results shown in figure 4 were obtained on samples of blood from a single donor. Results obtained on 36 other samples were qualitatively similar and all samples demonstrated thixotropic properties.

**Discussion**

Most observers of blood clotting are aware that the clotting time varies with mechanical shaking or with flow. The simple three-tube technique for determining clotting time, in which the third tube remains undisturbed for the final measurement, reflects this very point. In the present paper we have determined the quantitative relations between velocity gradient of flow and clotting time and have in addition studied the effects of flow on the rheological properties of the clot. The results show that clotting time is greatly reduced as rate of shear is increased and the evidence suggests the clotting process might take only a few seconds in blood exposed immediately to high rates of shear.

Some explanations are available to account for the decrease of clotting time with increase in velocity gradient. Diffusion phenomena might be greatly accelerated at lower viscosities corresponding to higher rates of shear, and/or possibly, the velocity gradient acts as a procoagulant in that it accelerates the rate of fibrin polymerization, perhaps via autothrombin C.\(^{12}\)

Associated with the changes in clotting time are the changes in the consistency of blood coagula formed at different velocity gradients. Blood coagula cast at increasingly higher rates of shear, show progressively lower viscosity. If the coagulation process proceeds at high enough rates of shear, no significant increase in viscosity can be observed. Coagula cast at low or intermediate rates of shear exhibit shear-thinning or thixotropic properties. Thixotropic properties of clots formed at zero-rate of shear have been noted previously by Scott Blair\(^{13}\) and by Deryagin and Yashin.\(^{14}\)

The physical, i.e., molecular and colloidal, structure of blood coagula can be deduced from their rheological flow curves. It is well known that in clots formed at zero rate of shear the dominant feature is a three-dimensional network of fibrin, the meshes of this network retaining the red cells. However, even a slight velocity gradient would lead to a certain degree of re-orientation of fibers and fragmentation of the fibrin network. It appears also that any further increase in the
rate of shear, used during the formation of coagulum, produces more and more fragmentation of the fibrin network; viscosity being less and less.

Coagula formed at high rates of shear have characteristics very different from those usually presumed to exist in a blood clot or thrombus. All platelets and fibrin are agglomerated in few large (macroscopic) particles, easily visible by naked eye; these particles do not affect viscosity of blood. It is known from earlier work\(^3\)\(^4\) that increasing aggregation of platelets follows an increase in the velocity gradient. Although this relationship corresponds to the transition from the red thrombus (or clot) to a white thrombus, the platelets themselves do not appear to influence viscosity of blood coagula. We have here, consequently, a spectrum of blood coagula, ranging from solid and gel-like, through highly viscous and thixotropic, to completely liquid forms. The latter, apparently, do not differ in viscosity from the uncoagulated or anticoagulated blood.

It might be expected that both the clotting time of blood and the consistency of clots are affected by certain diseases. Indeed, Macfarlane and Tomlinson\(^15\) observed that clots formed in vitro, at zero rate of shear, from blood of thrombotic patients are significantly stronger than clots formed from blood of healthy persons.

The new techniques, described in the present paper, should permit a study of clotting times and consistency and strength of clots over ranges of conditions analogous to the ones encountered in physiological and pathological states.

Summary

Clotting times of human blood and rheology of blood clots have been determined by means of a cone-in-cone viscometer, a ring-in-ring adapter, and a variable-frequency thromboviscometer. The last represents a variable-frequency version of the thrombelastograph.

The results of clotting time tests on blood samples from 118 donors indicate clearly that clotting time is a function of the velocity gradient at which clotting takes place. Clotting time decreases as the velocity gradient increases.

Consistency of blood coagula depends on the velocity gradient at which coagula are formed. Consistency, or viscosity, of coagula decreases when the velocity gradient increases. While the viscosity of clots formed at low rates of shear is of the order of 100 poises, coagula formed at intermediate rates of shear exhibit viscosity of a few decipoises. Viscosity of blood is not significantly altered if coagulation takes place at rates of shear near 400 sec\(^{-1}\).

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References


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