Blood Rheology: Effect of Fibrinogen Deduced by Addition

By Edward W. Merrill, D.Sc., Edwin R. Gilliland, D.Sc., T. S. Lee, B.S., and Edwin W. Salsman, M.D.

Previous studies have shown that human blood in the region of low shear stress (low flow rate) possesses a distinctive non-Newtonian relationship between shear stress \( \tau \) and shear rate \( \dot{\gamma} \) given approximately by the relation:

\[
\tau = \tau_y + b\dot{\gamma}^{\frac{1}{b}}
\]

where \( \tau_y \) is the yield stress and \( b \) is a constant. For this reason, data in the low shear region are conveniently plotted with the ordinate scale proportional to the square root of shear stress and the abscissa scale proportional to the square root of shear rate. Nearly straight lines result, the ordinate intercept of which is the yield stress. The yield stress is that value of shear stress below which no flow is possible.

These rheological relationships are the result of a reversible "structuring" of blood that occurs between red cells in the presence of fibrinogen. Although plasma proteins other than fibrinogen may play a synergistic role in the presence of fibrinogen, they have no effect in the absence of fibrinogen. Suspensions of red cells in physiological saline solutions of the plasma proteins other than fibrinogen show almost the same rheological characteristics as suspensions of red cells in plain physiological saline; they are almost Newtonian and totally lack yield stress. As shown elsewhere, the yield stress at constant fibrinogen concentration depends strongly on the hematocrit ratio, approximately according to the relation:

\[
\tau_y = a(H - H_c)^b
\]

where \( a \) is a constant (dependent on fibrinogen concentration), \( H \) is the actual hematocrit (normal 45 vol %); \( H_c \) is a critical hematocrit, between 2 and 7 vol % red cells.

In a previous paper, the importance of fibrinogen concentration was demonstrated by a method of subtraction. The present results concern the rheological properties produced by addition of fibrinogen to suspensions of red cells in saline, and more extensive measurements on the relationship between rheological properties and the concentration of innate fibrinogen found in native plasma.

Methods

Viscometry

A sensitive Couette viscometer employing an air bearing and a-c torque-to-balance loop was used in all of these experiments. By perfusion of the viscometer rotor with water at 37°C, and use of a plastic stator cup having low thermal conductivity, the temperature of blood samples was controlled at 37.0 ± 0.1°C in all experiments.

Blood Samples

Blood samples were obtained from healthy donors in the Massachusetts General Hospital Blood Bank and were drawn into standard ACD solution in Fenwall transfusion bags. Samples were centrifuged at 4°C to separate red cells and buffy coat from plasma, the buffy coat was removed, and the red cells were either resuspended in citrated plasma at a constant hematocrit of 40 vol% for rheometric testing, or were used in various nonplasma suspensions according to the following procedures.

Red Cell Washing

Red cells recovered by centrifugation were transferred into an equal volume of 0.15 M NaCl solution, gently mixed therein, and re-
TABLE 1

Characteristics of Blood Samples from Donors

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>Blood no. (MGH)</th>
<th>Original hematocrit</th>
<th>Fibrinogen* conc.</th>
<th>Type</th>
<th>Donor sex</th>
<th>( r_1' ) (^2 ) ( \times 10^{-2} )</th>
<th>( r_2 ) ( \times 10^{-2} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-2</td>
<td>00757</td>
<td>44</td>
<td>0.31</td>
<td>O+</td>
<td>M</td>
<td>0.17</td>
<td>2.90 X 10^{-2}</td>
</tr>
<tr>
<td>T-3</td>
<td>01001</td>
<td>42</td>
<td>0.36</td>
<td>A+</td>
<td>M</td>
<td>0.15</td>
<td>1.32</td>
</tr>
<tr>
<td>T-4</td>
<td>00887</td>
<td>39</td>
<td>0.31</td>
<td>A+</td>
<td>M</td>
<td>0.21</td>
<td>4.41</td>
</tr>
<tr>
<td>T-5</td>
<td>01431</td>
<td>43</td>
<td>0.25</td>
<td>O+</td>
<td>M</td>
<td>0.15</td>
<td>2.25</td>
</tr>
<tr>
<td>T-6</td>
<td>02284</td>
<td>38.1</td>
<td>0.28</td>
<td>O+</td>
<td>F</td>
<td>0.15</td>
<td>2.25</td>
</tr>
<tr>
<td>T-7</td>
<td>02281</td>
<td>43.2</td>
<td>0.41</td>
<td>O+</td>
<td>M</td>
<td>0.18</td>
<td>3.24</td>
</tr>
<tr>
<td>T-8</td>
<td>02815</td>
<td>42.5</td>
<td>0.19</td>
<td>O+</td>
<td>M</td>
<td>0.135</td>
<td>1.82</td>
</tr>
<tr>
<td>T-9</td>
<td>02818</td>
<td>33.5</td>
<td>0.27</td>
<td>A+</td>
<td>F</td>
<td>0.19</td>
<td>3.61</td>
</tr>
<tr>
<td>T-10</td>
<td>03329</td>
<td>46</td>
<td>0.31</td>
<td>O+</td>
<td>M</td>
<td>0.22</td>
<td>4.94</td>
</tr>
</tbody>
</table>

*Innate fibrinogen concentration in plasma of blood drawn into ACD bag.
†Square root of yield shear stress, at 37°C, hematocrit adjusted to 40.
‡Yield shear stress, at 37°C, hematocrit adjusted to 40.

**FIBRINOGEN**

A clinically administrable grade of human fibrinogen containing a substantial content of preservative salts (mainly phosphates) was used. This was either added directly to 0.15 M NaCl solution, in varying concentrations, or was dialysed at 4°C without stirring in standard dialysis bags against a solution containing 0.15 M NaCl and 0.0025 M trisodium citrate (3 volumes of dialysis fluid per volume fibrinogen solution). The dialysing bath was discarded after 12 hours, and the dialysis was repeated three times. The final content of clottable fibrinogen in the saline media, after admixture of red cells and viscometry, was determined by the method of Cullen and Van Slyke. In general, the concentration of clottable fibrinogen was about half of that added as dry material in the original preparation of the solutions, after allowing for the content of preservative salts. A part of the dry protein added to the saline remained in turbid suspension and was presumably inactive.

Master solutions of dialysed fibrinogen were diluted with 0.15 M NaCl to produce suspending media for red cells varying in analysable fibrinogen concentration from a maximum of about 1.2 g/100 ml to zero.

**Results**

1. EFFECT OF INNATE FIBRINOGEN CONCENTRATION

Table 1 and figure 1 present the rheological characteristics of the nine original blood samples (T-2 through T-10) altered from their in vivo state only in the following respects:

1. The samples having been drawn into transfusion bags containing ACD solution, the plasma proteins were diluted to about 85% of their in vivo concentrations; (2) in every case, the red cell concentration was adjusted to an hematocrit of 40%.

On the double square root plots of shear stress versus shear rate (fig. 1), almost linear curves are obtained for the data, in accordance with prior findings and no significant difference in shape (curvature) can be detected between type A+ blood (3 samples) and type O+ (6 samples). The ordinate intercept values in figure 1 which are the values of the square root of the yield stress, show an inconclusive trend toward a higher yield stress for type A+ cells than for O+ cells at equal concentrations of innate fibrinogen.* The direct values of yield stress, tabulated in table 1, are plotted vs. innate fibrinogen concentration in figure 5 below, curves 3 and 4, marked Innate: A+ and Innate: O+ and will be discussed subsequently in connection with the immediately following section on fractionated fibrinogen. One additional point from another experiment is indicated at the origin of figure 5, designated AF. This refers to a 16 year male subject with congenital afibrinogenemia. A sample of his blood was centrifuged. The supernatant was discarded and the procedure was repeated once more. Then the red cells, thus washed, were suspended in various media, described below, the hematocrit being adjusted in all cases to 40 vol%.

*Circulation Research, Vol. XVIII, April 1966
tested viscometrically directly after venipuncture, and the yield stress was found to be zero. Subsequent analysis for fibrinogen by the method of Ratnoff and Menzie showed zero content.

2. EFFECT OF ADDING FRACTIONATED FIBRINOGEN TO SUSPENSIONS OF RED CELLS IN PHYSIOLOGICAL SALINE SOLUTION

The purpose of the following experiments was to elucidate two questions: (1) the effect on rheology of salts, principally phosphates, that are present in clinically administrable fractionated fibrinogen, but are removed by dialysis; and (2) the effect of clinically administrable fibrinogen on the rheology of red cell suspensions according to concentrations of fibrinogen and red cells (A+ or O+).

It will be evident from the following data that, generally, red cells (A+ or O+) suspended in plain saline exhibit no yield stress whatever (as in the case of the subject with afibrinogenemia) and that addition of fractionated fibrinogen progressively increases the yield stress and transforms the blood into an increasingly non-Newtonian fluid, the general rheological characteristics of which, with type O+ red cells, are similar to those of native blood.

a) Dialysed Versus Undialysed Fibrinogen

From comparative tests using, respectively, fractionated fibrinogen as received, and the same after dialysis, on blood samples T4 through T7, which include one A+ type (T4) and three O+ types, it was concluded that the presence or absence of the dialysable salts in fibrinogen had no significant effect on any rheological property, in particular the yield stress. Typical data, by way of illustration, are shown in figure 2 (type A+) and in figure 3 (type O+). At the concentration of 0.59 (0.60) wt% fibrinogen, the respective curves on the double square root plot of figure 3 (O+ cells) for dialyzed and undialyzed fibrinogen virtually superimpose, and this was found to be the case for all other type O+ samples when the fibrinogen concentrations, as analysed, coincided within one or two per cent. Type A+ cells in the presence of added fibrinogen showed different relative positions of the curves according to whether the fibrinogen was dialyzed or not, but in an inconsistent manner. For example, in figure 2 the curve for 0.92 wt% undialyzed fibrinogen lies somewhat above the curve for 1.00 wt% dialyzed fibrinogen, whereas the curve for 0.46 wt%
Comparison of effects of dialysed and undialysed fibrinogen on type A* red cells. Double square root plots of shear stress versus shear rate. Red cells from sample T4 (A*) suspended in saline with indicated amounts of added undialysed fibrinogen (lower part of fig.) and added dialysed fibrinogen (upper part of fig.). Hematocrit 40, temperature 37°C.

Comparison of effects of dialysed and undialysed fibrinogen on type O* red cells. Double square root plots of shear stress versus shear rate. Red cells from sample T7 (O*) suspended in saline with indicated amounts of added undialysed fibrinogen (lower part of fig.) and added dialysed fibrinogen (upper part of fig.). Hematocrit 40, temperature 37°C.
undialysed fibrinogen is well below that for 0.50 wt% dialysed fibrinogen. We attribute these observations on the A sample T4 (fig. 2) to an unusual interaction of A* cells with fibrinogen, to be discussed below.

In all cases, whether the cells were type A* or O*, the yield stress did not vary systematically according to the presence or absence of dialysable salts, provided that the comparison is made at equal concentration of fibrinogen (and, of course, at the same hematocrit level of 40).

Table 2 summarizes the values of yield stress obtained for the red cell samples T4 through T7, with which both dialysed and undialysed fibrinogen were tested. It will be noted that for samples T5, T6, and T7 there is a substantial difference between the values obtained for undialysed fibrinogen at a concentration of 0.26 wt% and dialysed fibrinogen at a concentration of 0.29 wt%, but in these cases the yield stress was 16- to 100-fold lower than normal for blood with an hematocrit of 40, and so low as to be measurable only with an accuracy of about ± 25%.

At the higher concentrations of fibrinogen, where comparisons can be made between dialysed and undialysed fibrinogen (0.46 to 0.50, 0.92 to 1.00 for T4, 0.59 to 0.60 for T5, T6, and T7) it will be seen that the yield stress produced in dialysed fibrinogen is equal, within ± 20%, to that produced in undialysed fibrinogen.

b) Effect of Concentration of Added Fibrinogen According to Red Cell Type (A* or O*)

Supplementing the partial data of the kind shown in figures 2 and 3 (representative of the experiments on blood samples T4 through T7), experiments were done at six different concentrations of fibrinogen, using exclusively dialysed fibrinogen, on blood samples T8 (A*), T9 (O*), and T10 (O*). The double logarithmic plots are shown in figure 4.

It is immediately apparent (1) that in each sample with zero fibrinogen concentration, the control, there is zero yield stress; (2) that at concentrations up to about 0.14 wt%, there is not much effect, as compared with the control, (3) that above this concentration (0.14), yield stress increases rapidly as fibrinogen concentration is increased; (4) that the almost linear relations of square root of shear stress versus square root of shear rate for the two type O* samples (see also fig. 3) are the same in form as those for native blood (shown in fig. 1); and (5) that type A* cells in the presence of added fibrinogen show a different kind of rheology characterized by considerable curvature in the region between zero and about one inverse second of shear rate, followed by a straight section. It is important to note that, if data below 1.0 sec⁻¹ were not available, linear extrapolation of the data at higher shear rates would lead to intercept values 1.5 to 2 times higher than the actual ones, thus producing yield stress values too high by a factor of the square of 1.5 or 2. This same kind of curvature is also to be noted in the data for the sample of A* cells, T4, shown in figure 2.

The variation of yield shear stress with concentration of added dialysed fibrinogen for samples T8 to T10 as derived from figure 4, is shown in the last three columns of table 2, and complements the data for samples T4 to T7 already considered with reference to the effect of dialysable salts.

The graphs of figure 5 summarize the data in table 2 concerning the relation between yield stress and fibrinogen concentration for the seven red cell samples T4 through T10 (two being A*, five, O*). Data for both dialysed and undialysed fibrinogen are included.

It is obvious that at a given concentration of added fibrinogen a substantially greater yield stress is produced in a saline suspension of A* red cells than in a suspension of O* red cells (at the same hematocrit). Based upon these data, it is tentatively assumed that innate fibrinogen produces a higher yield stress with O* cells than with A* cells and the curves for yield stress versus innate fibrinogen have been drawn in accordingly through the scattered data as dashed lines (curves 3 and 4 of fig. 5).

Circulation Research, Vol. XVIII, April 1966
TABLE 2

Yield Shear Stress of Red Cell Suspensions at Various Concentrations of Dialysed and Undialysed Fibrinogen in Saline, at Constant Hematocrit of 40, Constant Temperature 37°C

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Blood number and type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T-4</td>
</tr>
<tr>
<td>0%</td>
<td>0.00</td>
</tr>
<tr>
<td>0.01%</td>
<td>0.00</td>
</tr>
<tr>
<td>0.02%</td>
<td>0.00</td>
</tr>
<tr>
<td>0.03%</td>
<td>0.00</td>
</tr>
<tr>
<td>0.04%</td>
<td>0.00</td>
</tr>
<tr>
<td>0.05%</td>
<td>0.00</td>
</tr>
<tr>
<td>0.06%</td>
<td>0.00</td>
</tr>
<tr>
<td>0.07%</td>
<td>0.00</td>
</tr>
<tr>
<td>0.08%</td>
<td>0.00</td>
</tr>
<tr>
<td>0.09%</td>
<td>0.00</td>
</tr>
<tr>
<td>0.10%</td>
<td>0.00</td>
</tr>
<tr>
<td>0.11%</td>
<td>0.00</td>
</tr>
<tr>
<td>0.12%</td>
<td>0.00</td>
</tr>
<tr>
<td>0.13%</td>
<td>0.00</td>
</tr>
</tbody>
</table>

* Undialysed fibrinogen solution.
Discussion

The experimental results confirm previous observations on the effect of fibrinogen on blood rheology and considerably augment them, the new findings being: a) The inconsequential influence of dialysable microionic salts (phosphates), present in fractionated fibrinogen, on the rheology of red cell suspensions, b) the substantially lower effect of added fractionated fibrinogen than of innate fibrinogen, at any level of concentration, and c) the apparently greater departure from Newtonian fluid characteristics with A* cells than with O* cells, at a given concentration of added fibrinogen.

Dialysable Salts

It was postulated that the dialysable salts, present in the fractionated fibrinogen as received, by systematic increase of the tonicity of the red cell suspension into the hypertonic region, might crenate the red cells and reduce their capacity for interaction with fibrinogen to form the aggregate structure which confers on blood its non-Newtonian characteristics. The data show clearly that the salts have no significant effect on the red cell suspension characteristics.
cell–fibrinogen interaction. Therefore it seems likely that clinical administration of fractionated fibrinogen with its preservative salts would have no advantage or disadvantage, compared to dialysed fibrinogen, with respect to changes of rheology of the patient’s blood.

**ADDED FIBRINOGEN COMPARED TO INNATE FIBRINOGEN**

No certain explanations are available for the observation that at least 0.14 g/dl of added fibrinogen must be present to achieve any significant yield stress, and that thereafter substantially higher concentrations of added fibrinogen than of innate fibrinogen are required to produce a given yield stress. It seems probable that part of the difference may be ascribed to alterations unavoidably produced in the original fractionation of whole plasma. Aside from the fact that the fibrinogen fraction contains clotting factor VIII (and is therefore administered in factor VIII deficiencies), there is reason to suspect that partial denaturation may occur leading to insolubility and inactivity of some of the protein. It was noted above that the actual content of fibrinogen found as clottable protein in the saline media was approximately half that which had been added, clearly demonstrating that a large percentage of the protein was incapable of activation by thrombin to yield fibrin. The saline suspensions of the fibrinogen fraction were turbid, whether dialysed or not. This signifies unequivocally the presence of suspended material in the micron size range (in contradistinction to molecular size of ca. 500 Å), and the point of uncertainty really lies in the question of whether a certain amount of the fibrinogen, existing as aggregates and therefore presumably not capable of adsorption on the red cell membrane, was nonetheless capable of thrombin activation. If this were the case, the aggregated protein, though ineffective in the process of aggregating red cells and thus incapable of influencing the low shear stress rheological behavior of the suspension, would appear in the total active fibrinogen count along with the single protein molecules.

**FURTHER SPECULATIONS ON FIBRINOGEN AND RED CELL INTERACTION**

It was originally believed that the low shear stress rheological characteristics of blood (as exemplified by the data presented above) were somehow related to rouleaux, on
two grounds: (1) rouleaux formation, as is well known, is strongly promoted by fibrinogen, and (2) the model upon which the double square root equation given above is based, as proposed by Casson, comprised the reversible association of suspended particles into rod-like ("rouleau" like) ensembles. This premise was found to be incorrect. In the first place, direct cinephotomicrographic observation of human blood, flowing in hollow glass and plastic fibers of internal diameters ranging from 50 to several hundred microns, at shear rates comparable to those of this study, failed to reveal any organization into rouleaux, except in the special case that the blood, having been maintained stationary in the tube for a few minutes, was allowed to creep slowly under a very small driving pressure. In the second place, re-examination of the model for the double square root equation showed that it should be completely inapplicable to suspensions, like blood, having a particle concentration as high as 40 vol%.

Cinephotomicrographic studies indicate, rather, that at low flow rates, red cells suspended in plasma form limited random, not rouleaux-like, aggregates and that these aggregates are continually "making and breaking," so that a dynamic equilibrium results with a spectrum of "aggregate" sizes, ranging from the individual red cell to clusters of 10 or more, depending on the level of shear stress. Further, it was found that when the flow of blood in hollow fibers is suddenly stopped, the red cells are initially "frozen into" a random three-dimensional structure out of which, over a period of a few minutes, rouleaux evolve.

From these observations we conclude that fibrinogen operates in some way with the red cell membrane to make every part of the superficial area of the red cell capable of sticking to any accessible part of an adjacent red cell surface. Whether fibrinogen makes one area of the red cell more "adhesive" than another does not seem easily determinable. One could argue that the eventual formation of rouleaux would suggest greater attraction per unit surface area along the peripheral faces of the biconcave red cell than elsewhere, except that, in the light of "membrane flicker" observed in red cells, it could equally well be argued that the red cells, originally organized in a random structure in static blood, have sufficient Brownian activity to jiggle into positions of maximum inter-surface contact (the rouleau) and will do so by thermodynamic necessity even if the specific attraction per unit area of red cell membranes is everywhere uniform.

Electron microscopy shows the fibrinogen molecule to be about 500 Å long with one central and two terminal balls about 65 Å in diameter. The red cell membrane, observed by electron microscopy, appears to consist of contiguous craters having diameters in the same range of size. From existing evidence it is impossible to decide with what kind of orientation the rod-like fibrinogen molecules might be adsorbed onto the red cell membrane, i.e., "end-on" or "lying down." One is intuitively (but perhaps erroneously) drawn to a model wherein the fibrinogen molecule is adsorbed "end-on" into crater-like sites by one of its terminal ball shaped elements, leaving the other terminal ball projecting outward for subsequent engagement with an absorbing site on the membrane of a neighboring cell. In effect, this model is one of monomolecular fibrinogen adsorption.

It is interesting to calculate, with respect to the quantities of fibrinogen used in the experiments cited above, how much fibrinogen would be needed to cover all hypothetical adsorbing sites on the red cell, arbitrarily placing these sites in a triangular array 65 Å between centers, each site therefore occupying 2150 Å². On the basis of 1 cm³ of blood at an hematocrit of 40 (therefore 4 × 10⁹ red cells, total projected red cell surface 0.6 × 10²⁰ Å², 0.6 cm³ plasma phase), 2.8 × 10¹⁰ sites are available. If all sites were occupied by fibrinogen (mol wt 340,000) absorbed end on, one finds that approximately 1.6 mg of fibrinogen would be needed. In the 0.6 cm³ of plasma phase, a clottable protein concentration of 0.5 g/100 ml (middle of
the range investigated) would correspond to 3 mg of fibrinogen.

Thus even in this limiting case, considerably more fibrinogen must be in free solution than adsorbed on red cell membrane. A more realistic model would consist of absorbing sites spaced at greater intervals than 65 Å, with probably a range of absorbing capacity for fibrinogen ranging from strongly tenacious to negligible. In view of the ease of removal of fibrinogen from red cell surfaces by repeated saline washing, and of the capability of thoroughly washed red cells to reduce the concentration of free fibrinogen in a saline solution upon mixing the two [by readsorption], it seems probable that a dynamic equilibrium exists between fibrinogen in free solution and fibrinogen adsorbed on the red cell membrane. The S-shaped curves of yield stress vs. free fibrinogen concentration (fig. 5) strongly suggest approach to saturation of available membrane sites at high (e.g., 1 g/100 ml) concentrations. The substantial differences between A* and O+ cells in the presence of a given amount of free fibrinogen may reflect significant differences either in the number of adsorptive sites per cell or the intensities of adsorption per site.

Summary

Viscometric measurements near zero shear stress were made on human blood and on suspensions of red cells in saline solutions of dialysed and nondialysed fibrinogen at 37°C at an hematocrit of 40. The principal viscometric parameter studied was the yield shear stress. The higher the value of yield stress, the greater is the viscosity defined as the ratio of shear stress to shear rate, in the region near zero flow, and the greater is the variation of viscosity with shear rate. The yield stress, zero in suspensions containing no fibrinogen, was found to increase with the fibrinogen concentration. At a given concentration of clottable fibrinogen (up to 1.20 g/100 ml) the yield shear stress was substantially greater in suspensions of red cells in their original plasma than in saline solutions of fractionated fibrinogen. Dialysis of the fractionated fibrinogen had no significant effect on the viscosity characteristics. For a given concentration of fibrinogen added to saline solutions, a higher yield stress was produced in the presence of A* cells than in the presence of O+ cells.

Acknowledgment

We gratefully acknowledge the continuing collaboration of Professor C. S. Draper, Mr. Philip J. Gilinson, Jr., and Mr. C. R. Dauwalter of the M.I.T. Instrumentation Laboratory. Mr. Gerard A. Pelletier carried out the numerous protein analyses.

References

Blood Rheology: Effect of Fibrinogen Deduced by Addition
Edward W. Merrill, Edwin R. Gilliland, T. S. Lee and Edwin W. Salzman

Circ Res. 1966;18:437-446
doi: 10.1161/01.RES.18.4.437
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1966 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/18/4/437

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/