Kinetics of Hemoglobin S Polymerization and Gelation Under Shear: I. Shape of the Viscosity Progress Curve and Dependence of Delay Time and Reaction Rate on Shear Rate and Temperature

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Polymerization and gelation of deoxyhemoglobin S makes red blood cells (RBCs) rigid and is the immediate basis of pathogenesis in sickle cell disease. Hence, characterization of hemoglobin S viscosity and its time-dependent development as RBCs pass through the microvasculature is important in understanding pathogenesis. Because RBCs and the intravascular milieu in vivo are subject to shear, the shear dependence of polymerization kinetics is also important. In steady-state cone-plate viscometry we find: (1) gelation under shear progresses exponentially with time; (2) shear markedly increases exponential rate and (3) shortens delay time independent of when in the delay time it is applied; (4) shear greatly decreases the temperature dependence of the exponential rate and delay time; (5) simultaneous with its acceleratory effect on polymerization, shear breaks down gel structure. We conclude that shear acts to accelerate gelation by breaking fibers and creating new growing ends, a process that occurs in addition to the homogeneous and heterogeneous nucleation of new fibers that occurs in the absence of shear. Fibers that break are part of a gel network rather than in free solution. The shear dependence of gelation rates means that the critical clinical issue, whether the delay time is long enough and gelation slow enough to permit deoxygenated cells to pass through the microvasculature before they rigidify, depends on in vivo shear rates as well as on degree of unsaturation and hemoglobin concentration.

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MATERIALS AND METHODS

Hemoglobin S was purified from blood (obtained from therapeutic exchange transfusions) of patients homozygous for HbS. Hemoglobin F and any previously transfused hemoglobin A were removed by chromatography on DE-52 anion exchange resin. Experimental samples were dialyzed into 0.1 mol/L potassium phosphate, pH 7.0, diluted to 14 mmol/L (heme) and deoxygenated under nitrogen near 0°C in a tonometer equipped with an absorption cell to check full deoxygenation (in the wavelength range 1,200 to 700 nm). Absence of oxyhemoglobin and methemoglobin was also confirmed on aliquots in a thin cell before and after viscometric experiments in the wavelength range 700 to 450 nm.

Samples were loaded cold into a Wells-Brookfield model RVT cone-plate viscometer (cone angle 1.565°) (Brookfield Engineering Laboratories, Inc., Stoughton, MA) in an anaerobic glove chamber. Each of many experimental runs on a sample was commenced with a temperature jump and continued through the delay time and subsequent rapid increase in viscosity. Upon completion of the run (usually shortly after excess viscosity reached 20 cp), the gel was melted by rapid cooling to near 0°C, still under shear. Low temperature was maintained for 10 minutes at 100 rpm (shear rate 384 seconds⁻¹) and then 10 more minutes at the speed of the subsequent experiment, under which conditions the results, as measured by delay time and progress curve rates, were repeatable over up to 90 runs. (Some deviation of results from the overall average in the first 2 to 4 runs was in any case smaller than the large shear-dependent changes). A thermistor attached to the viscometer chamber showed the temperature jump was 90% complete in 30 seconds and 99% complete in 2 minutes. Comparison of progress curves under shear from the onset and curves in which shear was begun only later (Fig 3 in reference 6) showed that the effective time of the temperature jump was about 50 seconds.

Data were collected with an oscillographic recorder and digitized into a PC with Sigma Scan (Jandel Scientific, Sausalito, CA). Records were measured up to the point at which the smooth curve evidently broke from its exponential shape, which was more than 20 cp in almost all experiments; for runs at 50 rpm curves were measured to full-scale stress, corresponding to 12 cp. The baseline (solution) viscosity was determined as the minimum viscosity (based on a running
average of 3 points) once the sample had warmed to the experimental temperature. This was subtracted from the total apparent viscosity (i.e., stress divided by strain rate, equal to true viscosity plus solid-like behavior) to obtain excess apparent viscosity as a function of time.

Progress curves were fitted by non-linear least squares using microTSP (Quantitative Micro Software, Irvine, CA). Results reported are from fits of the data from 0 to 20 cp (10 cp for 50 rpm), a range over which the curve is dominated by the fast exponential component (see below), but yet not high enough to exhibit deviation from exponential shape. Functions tested for fit to the data included a single exponential, the sum of two exponentials, a linear increase plus an exponential, and a quadratic plus an exponential. Each exponential was characterized by a delay time (the time at which the fitted excess viscosity, for that exponential if more than one, reached 1 cp) and by a rate constant. The delay times reported are after correction for the time of the temperature jump. Most delay time data are reported as inverse delay times because this is a measure of reaction rate.

In experiments designated “sparing” (as opposed to continuous shear), the temperature jump was performed in the absence of shear, which was instituted only later, after a “sparing” interval without shear.

RESULTS

The shape of the progress curve. Figure 1 shows a typical progress curve, characterized by a delay time followed by a rapid and approximately exponential increase in excess viscosity. The data are fitted to a single exponential and to the sum of two exponentials. The same results are shown semilogarithmically in the inset, showing the improvement in fit when two exponentials are used.

Although all data showed a significant improvement in fit when two exponentials were used, the parameters obtained from the slower exponential were variable. In addition, the rates obtained from single exponential fits were dominated by the fast component of the two exponential fits. Thus, the basic results, including the shear and temperature dependences of rates, are similar whether shown in terms of the rates of single exponentials or of the fast term of double exponentials. The use of other functions plus an exponential usually gave poorer fits than double exponentials and suffered from the same variation in the additional (i.e., linear, quadratic, or slow exponential) term from run to run. For these reasons the results are reported fitted to single exponentials.

Figure 2 shows the repeatability of results for a series of runs under the same conditions on a single sample.

Progress curves and delay times under continuous shear and with “sparing.” Figure 3 shows a series of progress curves at the same shear rate along with their fitted exponentials. In one run shear was continuous from the time of the temperature jump through the exponential growth stage. In the other (“sparing”) runs the temperature jump occurred in the absence of shear and the period of no shear then continued through a portion of the delay time, after which shear was instituted. The existence of a “sparing” time under no shear.
shear has a large effect on the subsequent delay time under shear. However, there is little or no effect of “sparing” on exponential rate.

In the right-most curve of Fig 3, shear was withheld until the delay period was over and substantial excess viscosity existed, evident upon commencement of shearing. Under shear this excess viscosity decreased at first and then showed the usual exponential increase. The initial excess viscosity represents gel structure and its early decrease represents the shear-induced breakdown thereof. Thus, shear has two effects that can occur simultaneously: breakdown of structure that contributes to viscosity and acceleration of gelation. When substantial structure exists, the first effect predominates. When lesser degrees of structure exist it may be expected that, although excess viscosity does not decrease upon institution of shear, its initial rate of increase is diminished, thus producing exponential progress rates that are initially slower than the rate caused by polymerization alone. (There is a suggestion of this in the regressions of Figs 4 and 5, below).

Figure 4 shows delay times and exponential rates at 10 rpm (38.4 seconds⁻¹) under continuous shear and, in “sparing” experiments, their dependence on the length of the time at no shear. The exponential progress rate is not significantly affected by the presence of a “sparing” time or by its length. In contrast, as previously reported⁶,⁷ and shown here for comparison, the delay time under shear is markedly shortened as “sparing” time under no shear increases. The y-intercept of the linear regression of time at shear against time at no shear is the (extrapolated) delay time were shearing continuous. The x-intercept represents, also by extrapolation, the time required for the temperature jump, confirmed by direct temperature measurement. The y-intercept of the regression is the true delay time under shear. It is slightly shorter than the delay time measured under continuous shear (arrow) because the latter is spuriously lengthened as a result of the time required (under shear) for the temperature jump. (The difference between the two delay times, 40 seconds, is a measure of the time required for the temperature jump, confirmed by direct temperature measurement). The x-intercept is the delay time were there no shear at all.

Figure 5 shows regressions of exponential rate against time at no shear for all shear rates examined. As for 10 rpm in Fig 4, the rates vary insignificantly with “sparing” time. The small decrease that does occur as “sparing” time increases could, if real, result from the mechanism suggested in connection with Fig 3: when “sparing” times are long there is an initial breakdown of structure when shear is started, contributing a decrease in gel viscosity that diminishes the observed rate of exponential increase.

The dependence of exponential rate and inverse delay time on shear rate. Although the exponential progress rate under shear is independent of prior shearing events in the delay time, it depends strongly on the rate of shear during the period of its measurement, shown in Fig 6. Exponential rate increases markedly as shear rate increases. This is similar to the effect of shear on the inverse delay time, shown in Fig 7, which summarizes and extends previous results.⁶,⁷ In both Figs 6 and 7, the effect of shear (ie, the slope of kinetic rate against shear rate) diminishes at high shear rates, indicating that ki-
Fig 5. The lack of effect of shearing or its absence during the delay time on exponential progress rate under shear at different rates. Exponential progress rates are plotted semi-logarithmically as functions of "sparing" time at no shear for different shear rates: 8.6 seconds^{-1}; \( \triangle \) 19.2; \( \square \) 38.4; \( \diamond \) 76.8; \( \triangledown \) 192; • 3.84 (no "sparing" experiments done). Points on the ordinate represent runs under continuous shear ("sparing" time = 0). The regressions are linear (they necessarily have small curvatures in the semi-logarithmic plot; this form of plot, however, permits better comparison of the ratio of slope to magnitude of the exponential rate). The slopes are low, indicating that the dependence of progress rate on "sparing" time is not significant. The y-intercepts provide the extrapolated progress rates for continuous shear, which are similar to the averages under continuous shear. The regressions are for points with "sparing" time at no shear and are drawn up to the delay time at no shear obtained from the x-intercepts "sparing" plots as in Fig 4.

Fig 6. The effect of shear rate on the rate of exponential progress at 20°C. Increasing shear rate from 3.84 to 192 seconds^{-1} increases the rate 20-fold. Exponential rates, obtained in three ways from the data in Fig 5, are shown at each shear rate: •, exponential rate under continuous shear; \( \square \), rate obtained by extrapolation of "sparing" data to no "sparing" time (Fig 5 y-intercept): \( \triangle \), rate obtained by extrapolation to "sparing" delay time at no shear (ie, right extreme of Fig 5 regression lines). The different measurements give basically the same result. Standard deviations for continuous shear data were 11% of the corresponding rates.

Discussion

We now consider how shear affects gelation kinetics and produces the experimental results reported. The problem has two parts: on what process and/or stage of gelation does shear act? What is the mechanism of the action?

Shear and the double-nucleation model. We first assume that the double-nucleation model\(^8,9\) applies under shear, and then consider modifications to it that might be needed. The assumption is reasonable in view of similarities in kinetics:

Comparison with kinetic results by non-invasive methods. Table 1 compares rates in this work in the absence of shear (by "sparing" extrapolation) with rates by light scattering.\(^5,9\) The comparison supports use of the "sparing" extrapolation to obtain kinetic data at no shear.
Fig 8. The dependence of inverse delay time and exponential rate on shear rate, plotted logarithmically to show power dependence. The upper solid regression line and the points near it represent exponential rates. The lower solid regression line and the points near it represent inverse delay times. In each of these groups circles (○) are directly measured values (continuous shear) and squares (□) are extrapolations to the y-axis from "sparing" experiments. The regression lines are for continuous shear (circles). Triangles (▲) are x-axis extrapolations representing values there were no shearing during the delay time. For exponential rate regression they are the same as other data but for inverse delay times they reflect extrapolated delay time at no shear, shown at the bottom of the figure (dash-dot regression line). The slope of the regression for exponential rate is 0.80 ± .06 (SD) and that for inverse delay time under shear is 0.61 ± .06 for all points; if the point at lowest shear rate is omitted, the slope (dotted line) is 0.72 ± .04. The extrapolated delay time at no shear should be independent of shear rate. It is nearly so, supporting the validity of this method of its determination.

(1) there is a delay time and exponential progress under shear as in its absence;1,2,13,15 (2) shear has the same acceleratory effect during the "delay stage" and the later "growth stage," consistent with the basic identity of mechanisms in the two "stages" (which differ only in respect to whether the amount of polymer present is detectable); and (3) the activation energies for delay time and exponential rate are the same under shear, as they are in its absence.14,16

Under double nucleation the first fibers are formed by homogeneous nucleation in bulk solution. As the mass of fibers increases, the dominance of homogeneous nucleation is replaced by a predominance of fibers created heterogeneously (on the surface of existing fibers) and reaction progress becomes exponential. Conversely, the observation of exponential progress is evidence that heterogeneous nucleation

Table 1. Comparison of Present Results With Other Data

<table>
<thead>
<tr>
<th>Reference</th>
<th>Method</th>
<th>T</th>
<th>pH</th>
<th>Conc</th>
<th>t_d (sec)</th>
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<td>7.0</td>
<td>1</td>
<td>14.1, 1100</td>
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<tr>
<td></td>
<td>Light scat</td>
<td>20</td>
<td>7.0</td>
<td>.1</td>
<td>14.1, 620</td>
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<tr>
<td></td>
<td>Light scat</td>
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<td>7.0</td>
<td>.1</td>
<td>14.1, 1050</td>
</tr>
<tr>
<td>Ferrone et al</td>
<td>Light scat</td>
<td>25</td>
<td>7.35</td>
<td>.15</td>
<td>14.08, 4100</td>
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</table>

The present data at no shear by "sparing" extrapolation are consistent with the light-scattering data of Briehl and Christoph.4 The data of Ferrone et al.18 were corrected from their experimental conditions using their activation energies to correct to 20°C and the supersaturation equation19 to correct for concentration and pH (with n = 35 and the ratio of c_{dep} at pH 7.35 to that at pH 7.0 as 1.12).23; corrections made this way are highly sensitive to changes in the correction parameters, so the calculation shows potential but not proven consistency.
dominates homogeneous. Once formed, fibers grow by non-cooperative addition of monomers.

Progress curves provide two observable parameters (A and B) under the basic progress equation of the double-nucleation model:

\[ Q(t) = A[\cosh(Bt) - 1] \]  

(1)

where \( Q(t) \) is the time-dependent concentration of hemoglobin polymer, \( A \) is an amplitude, and \( B \) expresses the progress rate. Once heterogeneous nucleation dominates, equation (1) is well approximated by an exponential,

\[ Q(t) \approx A \exp\left(\frac{Bt}{2}\right) \]  

(2)

The results of Ferrone et al.\(^{16}\) under conditions similar (except for shear) to the present suggest that the approximation is valid in these studies. The fact that we observe exponential progress (rather than quadratic, as occurs very early when homogeneous nucleation dominates) supports the applicability of equation (2). Therefore, our observed exponential rates provide values of \( B \).

**On what process does shear act to accelerate gelation?** The delay time, \( t_d \), depends on both B and A, but principally on the former,\(^{16}\) as can be seen from the simulated progress curves of Fig 11. Hence, we consider the determinants of B and assume those of \( 1/t_d \) are largely the same. B depends on heterogeneous nucleation, monomer addition, and homogeneous nucleation. The basic equation\(^5\), is,

\[ B = \sqrt{k_c(\gamma_0c_0 - \gamma_c)c_0} \]  

(3)

where \( k_c \) is the rate constant for monomer addition, \( c_0 \) and \( c_\text{a} \) are total concentration and \( c_\text{a} \) (the monomer concentration in equilibrium with the gelled phase), respectively, \( \gamma_0 \) and \( \gamma_c \) are the corresponding activity coefficients, and \( \gamma_0c_0 \) and \( \gamma_c \) are, respectively, the heterogeneous and homogeneous nucleation rates at concentration \( c_0 \). Heterogeneous nucleation is the most critical factor for B because the large cooperativity of nucleation means that small changes in monomer concentration or solution conditions produce large changes in B. The correction of \( g_0 \) by the concentration dependence of homogeneous nucleation, \( \delta g_0/\delta c_0 \), is not significant under the conditions of the present experiments,\(^9\,11\) so that equation (3) can be replaced by,

\[ B \approx \sqrt{k_c(\gamma_0c_0 - \gamma_c)c_0} \]  

(4)

B also depends on the net (‘on’ minus ‘off’) rate \( [k_c(\gamma_0c_0 - \gamma_c)c_0] \) at fiber ends. However, the effect of shear on collision rates is small compared with diffusional effects and hence \( k_c \) should not change with shear. Furthermore, monomer addition is first order in monomer concentration and hence large effects of the kind observed caused by shear do not seem likely as a consequence of any action of shear on \( k_c \).

And \( c_\text{a} \) shows the properties of an equilibrium phase transition and thus ought not depend on shear. Therefore, the net ‘on’ minus ‘off’ rate should not depend on shear, nor B on shear through it.

Consequently, B and its shear dependence depend primarily on the heterogeneous nucleation rate or on a process that, under the model’s equations, is not distinguishable from heterogeneous nucleation.

**Possible mechanisms for the action of shear.** The double-nucleation model assumes equilibrium nucleation, i.e., the critical nucleus is in rapid equilibrium with monomers in solution through a series of bimolecular steps. The model also assumes that the structure of the nucleus is essentially the same as that of a small piece of fiber, apart from end effects (diminution in the number of intermolecular contacts per monomer as a result of the small size of the nucleus). Mechanisms by which shear might alter heterogeneous nucleation are: (1) if nuclear structure were altered, the equilibrium between nuclei and monomers would change; (2) if the bimolecular equilibria were altered, the overall equilibrium (as well as nuclear size) would also change; (3) equilibrium nucleation might be incorrect, requiring change in the basic model, at least under shear. However, it does not seem likely that moderate rates of shear would alter heterogeneous nucleation rates through such effects on structure and/or chemical equilibria.

A more plausible explanation is that shear creates new fibers by breakage of existing ones and hence accelerates gelation by producing more fiber ends at which polymerization proceeds. Although this is not a nucleation process, measurements of polymer mass alone do not distinguish it from the formation of new fibers by heterogeneous nucleation. Hence, the hypothesis of breakage is consistent with the argument that shear enhances a process that is represented by \( g_0 \), the heterogeneous nucleation rate parameter. The hypothesis of fiber breakage is consistent with the observation that shear can reduce viscosity during gelation even as polymerization progresses, as illustrated by the period of decreasing viscosity in the right-most progress curve of Fig 3.
Fiber breakage. Fiber breakage has previously been suggested and discussed as a possible factor in gelation kinetics. However, these approaches assumed fibers exist in free solution subject to shearing forces, contrary to the observation that gels are solid-like and have many stable cross-links forming a well-defined network (see below); and/or they assumed significant spontaneous breakage (ie, independent of shear) at variance with the observation that fibers break spontaneously only very rarely.

Because gels are solid-like, gel structure must break under the large displacements induced by steady-state shear. It is highly probable that breaks occur in fibers rather than exclusively in stable cross-links. In consequence, new ends at which growth can occur are formed and the reaction proceeds more rapidly than in the absence of shear.

If the heterogeneous nucleation rate is replaced by the sum of new fiber creation by heterogeneous nucleation and by fiber breakage, the formal equations of the double-nucleation model suffice to include fiber breakage with the introduction of a term for it. In the absence of shear, the rate of formation of new fibers by heterogeneous nucleation is $Q(t)g(c)$, where $g(c)$ is the concentration-dependent heterogeneous nucleation rate and $c$ is the concentration of monomers in solution. If there is shear-induced fiber breakage, the simplest assumption is that the rate of breakage and new fiber-end creation is $Q(t)g(c)$, where $b(G)$ is a rate parameter for breakage that depends on shear rate, $G$. Thus, for double nucleation with shearing, $g(c)$ is replaced by $g(c) + b(G)$, as pointed out by Eaton and Hofrichter. The solution for reaction progress still gives equation (1). But $B$, previously given by equation (4), now depends on breakage as well as true heterogeneous nucleation,

$$B = \bar{b} k_{c} \left[ (\gamma c_{0} - \gamma c_{c})(g_{0} + b(G)) \right]$$  

$$B = \frac{1}{2} \ln k_{c} + \ln (\gamma c_{0} - \gamma c_{c}) + \ln (g_{0} + b(G)) \right]$$  

Differentiating equation (6) with respect to $\ln G$ gives the power dependence of exponential rate $B$ on shear,

$$\frac{\partial \ln B}{\partial \ln G} = \frac{1}{2} \frac{b(G)}{g_{0} + b(G)}$$  

At very low shear rates true heterogeneous nucleation greatly exceeds breakage ($g_{0} \gg b$). Then $\partial \ln B / \partial \ln G \cong 0$ and there is no power dependence of progress rate on shear rate. (Such a diminution of the effect of shear may begin to occur at the lowest shear rate observed in Fig 8 for inverse delay time). At high shear, true heterogeneous nucleation is negligible in comparison with breakage ($g_{0} \ll b$), and $\partial \ln B / \partial \ln G \cong [d \ln b(G) / d \ln G] / 2$. In our results the power dependence of $B$ on shear is about 0.8, so that breakage, $b$, is proportional to the 1.6 power of shear rate.

Breakage and mechanism. Under a simple assumption that the rate of fiber breakage ($b$) is proportional to shear rate, equation (5) indicates that $B$ will be proportional to the square root of shear rate for high shear rates ($b \gg g_{0}$). Because our results show a higher power dependence of $B$ on $G$, the relations between shear rate and breakage require further consideration.

The total polymer mass concentration, $Q$, is the number concentration of fibers, $n$, times average fiber length, $L$,

$$Q = nL$$  

The rate of new fiber formation, $dn/dt$, can be obtained from the time, $t_{l}$, required for fibers (which are assumed to break in half) to grow from broken length $L$ to length $2L$, at which they again break,

$$t_{l} = \frac{L}{k_{2}}$$  

where $k_{2} = k_{c} (\gamma c_{0} - \gamma c_{c})$ is the net rate of fiber elongation. The breakage frequency is then $1/t_{l} = k_{2}/L$ and the breakage frequency per unit volume is obtained by multiplying by $n$ to give,

$$\frac{dn}{dt} = n \frac{k_{2}}{L}$$  

The breakage frequency parameter, $b$, is frequency per mass concentration of polymer, $Q$, so that, using equations (8) and (10),

$$b = \frac{1}{Q} \frac{dn}{dt} = \frac{k_{2}}{QL}$$  

The additional reasonable assumption that $L$ and $\bar{L}$ are proportional leads to the conclusion that $b$ is proportional to $1/L^{2}$.

$$b = \text{const} \times \frac{1}{L^{2}}$$  

The dependence of $b$ on shear rate can then be ascertained from the dependence of $L$ on shear rate. Two basic models might be applicable to the present results: fibers may be in free solution or they may be cross-linked in a solid-like gel.

Levinthal and Davison have characterized the dependence of the length of breakable fibers in free solution on shear rate: tensile force on a fiber in the shear field (at any orientation to the field, including the 45° optimum for breaking) is proportional to shear rate and to the square of fiber length. (This is because the breaking force depends on $L$ for two reasons: (1) fluid velocity at fiber ends increases proportional to $L$; and (2) the length of fiber exposed to shearing force is $L$). Hence, for fixed critical breaking force, the product $GL$ is constant and $L^{2}$ is therefore proportional to $1/G$. According to equation (12), $b$ is then proportional to $G$. Under equation (5) $B$ is therefore proportional to the square root of $G$. This is at variance with our data and suggests that a model of free fibers in solution is not adequate.

If, alternatively, the system is a solid-like gel, shearing induces breakage and the consequent existence of solid layers sliding along each other. The frictional force of shear is then applied only to the ends of fibers at the moving interface between layers. Therefore, force is proportional only to fiber length (mechanism [1] above); the remainder of the fiber experiences no force from the solvent (mechanism [2]). At critical breaking force, the product $GL$ is constant and fiber length is proportional to $1/G$. Therefore, $b$ is then proportional to $G^{2}$. Under equation (5), the observed rate, $B$, is then proportional to $L$.

In our results, $B$ depends on the 0.8 power of $G$, consistent with a major contribution from breakage of a solid-like gel to the shear-induced acceleration of gelation.
The mechanism of polymerization acceleration by shear-induced breakage of a solid-like gel is consistent with rheologic observation of yield stresses\(^{20}\) and with microscopic observations of gel structure\(^{10}\) showing that cross-links and hence solid-like structure form very early in gelation. Hence, the breakage-dependent effects of shear ought to be similar no matter when in the delay time or growth “stage” the shear is applied, as we observe.

We assumed the existence of a critical breaking force\(^{22}\) that defines the (shear-rate-dependent) length at which fibers break. In fact, thermal effects help govern breakage length and rate, producing a distribution in each. Thus, our analysis is a first approximation. However, particularly in the case of a solid gel, deformation increases quickly under shear and this cause of breakage can be considered to be dominant. If thermal breakage predominated, forces caused by shear would lower the activation barrier but not induce breakage and breakage rates would then depend exponentially on shear rate,\(^{19}\) rather than in accordance with a power law, as shown in Fig 8.

The temperature dependence of rates under shear. The present results can be described as showing a decreased shear dependence of kinetic rates as temperature increases or, equivalently (and more usefully here), as a decreased temperature dependence under shear.

In the absence of shear, the temperature dependence, and hence activation energy, of gelation is largely caused by the polymeric nature of and the many intermolecular interactions in the nucleus. In contrast, breakage caused by shear, the predominant cause of new fiber creation at high shear (as is evident from the many-fold increase in rates under shear over those in its absence), shows only a small temperature dependence.

Differentiation of equation (6) with respect to 1/T gives the Arrhenius activation energy for B and breaks it into its components parts,

\[
\Delta H^a = \frac{1}{2} \left[ \Delta H^c_k + \Delta H^c_w \left( \frac{1}{S_k} - 1 \right) + \Delta H^c_b \frac{g_0}{g_0 + b(G)} + \Delta H^c_h \frac{b(G)}{g_0 + b(G)} \right]
\]

(13)

where \(\Delta H^a\) indicates activation energy and subscripts \(k\), \(g_0\) and \(b\) refer to monomer addition, heterogeneous nucleation, and breakage, respectively; \(\Delta H^c_w\) is the equilibrium enthalpy; and \(S_k = g_0\gamma / f_s\) is the supersaturation ratio expressed as activity. \(\Delta H^c_k\), representing the noncooperative addition of monomers, should be small compared with the activation energy for nucleation. \(\Delta H^c_w\) is approximately 8 to 12 kJ/mol and \(S_k\) is in the range 2 to 3, so that the second term is small (about 8 kJ/mol). The magnitude of \(\Delta H^c_b\), the temperature dependence of breakage caused by shear, is unknown but must be small because the total activation energy under shear is small. Therefore, at very low shear rates (when \(b < g_0\)), the large observed activation energy is a result of heterogeneous nucleation. At high shear rates (\(b \gg g_0\)), the small activation energy reflects the first, second, and fourth terms.

Although the data do not offer enough information to test equation (13) quantitatively, they are consistent with it. In the absence of shear the activation energy for B can be taken as approximately equal to that for inverse delay time, 185 kJ/mol (comparable with data in the literature). The decrease to 38 kJ/mol under shear at 38.4 seconds\(^{-1}\) results from the decrease in \(g_0/(g_0 + b)\) and hence in the third term of equation (13) as \(b\) becomes larger than \(g_0\). That \(b\) is much larger than \(g_0\) can be deduced from equation (7) and Fig 8. Equation (7) states that the power dependence of B will approach its maximum and be constant for large \(b\) (ie, \(b/(g_0 + b) \cong 1\)). Fig 8 shows the power dependence to be constant at (and well before) shear rate 38.4 seconds\(^{-1}\). Thus, most of the 185 kJ/mol activation energy in the absence of shear is caused by heterogeneous nucleation while the 38 kJ/mol that remains under shear arises principally from the first, second, and fourth terms, confirming the prediction that they are small.

Fieschko et al\(^{14}\) used an oscillating sphere viscometer (hence a shear rate that was variable with both distance from the sphere and time) and found activation energies of 100 and 79 kJ/mol for doubling time (ie, exponential rate) and delay time, respectively. Their approximate shear rate is given as 2 seconds\(^{-1}\). This is intermediate between our rates and no shear, and their activation energies are similarly intermediate, supporting the conclusion that activation energy decreases as shear rate increases.

Other possible actions of shear. Shear decreases apparent viscosity in fully gelled preparations,\(^{23,24}\). The initial period of decreasing viscosity in the right-most progress curve of Fig 3 shows that shear-induced breakdown also occurs during polymerization. Therefore, viscosity need not remain proportional to the mass concentration of polymer as gelation progresses and some of the effects we observe may be modified by shear-induced breakdown and consequent nonlinear relations between apparent viscosity and polymer concentration (however, constancy of shear rate probably minimizes any lack of proportionality).

Once polymers form they can interact and form new structures. Shear may affect these and therefore the rheology that depends on them. Alignment and tactoid formation\(^{25}\) is enhanced by shear\(^{18}\) and can decrease viscosity.\(^{26}\) Shear can also accelerate gel cross-linking\(^{27}\) and might increase apparent viscosity through this mechanism. Finally, shear might pack spherulitic domains\(^{5,17,28}\) thereby altering viscosity.

Possible clinical implications. The primary conclusion from these results is that shear greatly accelerates hemoglobin S gelation, even at moderate shear rates, and it decreases the temperature dependence of the kinetic rates. The basis of this action is shear-induced breakage of fibers in the cross-linked and solid-like hemoglobin S gel.

The transit time for RBCs to pass the microvasculature is critical for induction of crises.\(^{24}\) If cells pass during the delay time, when they are still highly deformable, obstruction will be avoided. But if factors such as low oxygen tension or high hemoglobin concentration shorten the delay time, or if transit time is increased, obstruction will occur.

If shear within RBCs is sufficient to break fibers, it will contribute to shortening the delay time and accelerating gelation, and hence might be a major factor in governing the occurrence of microvascular obstruction and sickle cell crises. However, shear rates can only be estimated.
Intraerythrocytic shear might arise from transmission of shearing action in the surrounding plasma or from RBC deformation caused by a variety of causes. Shear can be transmitted to the cell interior by tank-treading. However, this occurs in large vessels only when the suspending medium viscosity is well above that of normal plasma. Model systems indicate that in the microvasculature it occurs normally but produces intraerythrocytic shear rates an order of magnitude or more smaller than the 800 seconds\(^{-1}\) calculated at the vessel wall under the assumption of a parabolic velocity profile. Estimates of intraerythrocytic shear caused by transient cellular deformation are even more uncertain. Factors such as RBC crowding, collisions between RBCs, and collisions between RBCs and vascular bifurcations and walls produce deformations. But the lack of information on the time scale as well as the extent of deformation precludes estimates of the degree of shear (or turbulence) and hence of the potential for breakage of HbS fibers. Thus, the extent to which intraerythrocytic shear may shorten delay time and accelerate gelation kinetics in vivo cannot be ascertained from the data available.

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