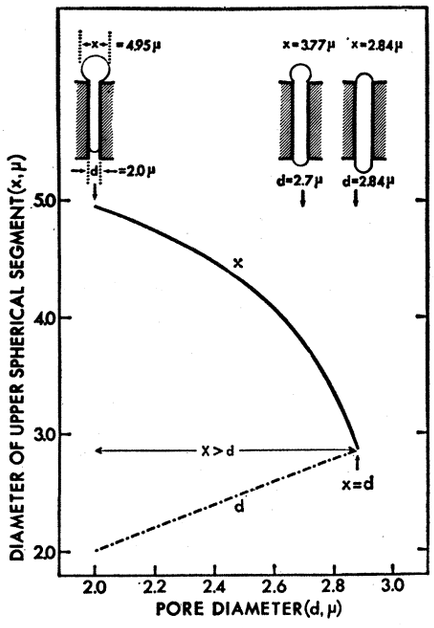

Fig. 4. Theoretical calculation of critical pore diameter through which an RBC with a volume of 100 μ^3 and a surface area of 150 μ^2 can pass without membrane stretching. The diameter (x) of the upper spherical segment of the RBC is reduced as the filter pore diameter (d) is increased. The curve for x intersects the dotted line d when the RBC can pass through the pore without tensile strain. Changes in x at three selected d values are shown in the upper part of the figure.

out tensile strain. This is in agreement with our experimental findings and with the results reported by Burton (*10*).

The ability of RBC's with a mean major diameter of over 7 μ (Fig. 1) to pass through smaller pores obviously necessitates cell deformation (Fig. 4). In this connection, it should be noted that RBC's hardened with acetaldehyde (*1*), although having normal size and shape, cannot be recovered in the filtrate even when sieves with 6.8-μ pores are used.

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6. Eagle-albumin solution contains 0.68 g of NaCl, 0.04 g of KCl, 0.014 g of $NaH_2PO_4 \cdot H_2O$, 0.22 g of $NaHCO_3$, 0.02 g of $CaCl_2$, 0.017 g of $MgCl_2 \cdot 6H_2O$, 0.1 g of dextrose, and 0.25 g of human serum albumin per 100 ml.
7. Ringer solution contains 0.86 g of NaCl, 0.03 g of KCl, and 0.033 g of $CaCl_2$ per 100 ml (Ringer's Injection, Abbott Laboratories, Chicago, Ill.).
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11. Carried out under Army contract DA-49-193-MD-2272 and supported by PHS research grant HE 06139 and by several private donors, including Mrs. George W. Perkins, the Alexander Angus McDonell, Jr., Foundation, and Mrs. Alan M. Scaife.

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Blood Viscosity: Influence of Erythrocyte Deformation

Abstract. *Suspensions of canine and human erythocytes hardened with acetaldehyde differ from the suspensions of normal erythrocytes with respect to their rheological behavior. Normal erythrocytes can be packed by centrifugation so that the sediment volume is nearly 100 percent cells, but the hardened erythrocytes (RBC's) can be packed only to approximately 60 percent cells. At the same cell percentage the viscosity of the hardened RBC suspension is higher than that of the suspension of normal erythocytes. An increase in shear stress deforms the normal erythocytes and lowers the suspension viscosity, but has no influence on the viscosity of the hardened cell suspension. In blood with high cell percentages, the shear deformation of normal RBC's plays an important role in reducing viscosity and facilitating flow at high shear stresses.*

There is ample evidence that normal erythrocytes (RBC's) can be deformed (*1*, *2*), but the role of RBC deformation in determining the flow properties of the blood has not been clearly established. In our experiments, we compared the viscometric behavior of suspensions of hardened RBC's and normal RBC's in an attempt to analyze the relation between RBC deformability and suspension viscosity.

Human and canine RBC's were washed with 0.9 percent NaCl solution and hardened in a 2 percent (weight to volume) acetaldehyde solution in 0.9 percent NaCl (*p*H buffered to 7.4) (*3*). After 1 month of hardening, the RBC's were washed with and then suspended in 0.9 percent NaCl or distilled water.

During the first week of the hardening process, the RBC suspension in acetaldehyde showed a progressive increase in the hematocrit reading as obtained by centrifugal packing. Since the RBC size determined by microscopic observation as well as with an electronic particle counter was unchanged, the rise in hematocrit suggested a reduction in the completeness of centrifugal packing rather than RBC swelling. The degree of trapping of the fluid medium in the packed cell column was determined by a dilution technique (*4*) with radioactive macromolecules (albumin-I^{131}, dextran-C^{14}, or polyvinylpyrrolidone-C^{14}). After centrifugation at 15,000*g* for 5 minutes or at 1,500*g* for 30 minutes, the volume fraction of the packed cell column occupied by hardened RBC's averaged only 0.60 (standard error of mean = 0.01); that is, 0.40 of the column consisted of trapped fluid. Centrifugation at 15,000*g* for 30 minutes increased the volume fraction occupied by hardened RBC's only slightly (0.62). In contrast, centrifugation of normal RBC suspensions under these conditions gave packed columns with RBC's occupying 0.95 to 0.97 of the volume. The value of 60 percent packing for hardened RBC's agrees closely with that calculated on a theoretical basis (*5*).

Suspensions of normal and hardened RBC's were made to contain various

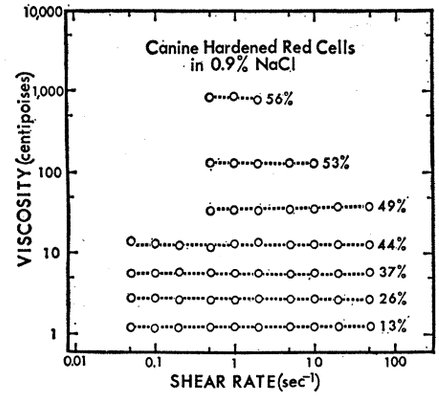

Fig. 1. A log-log plot of the relationship between viscosity and shear rate for suspensions of hardened canine RBC's in 0.9 percent NaCl. The cell percentages for the suspensions are shown for each curve. The viscosity values are essentially independent of the shear rate.

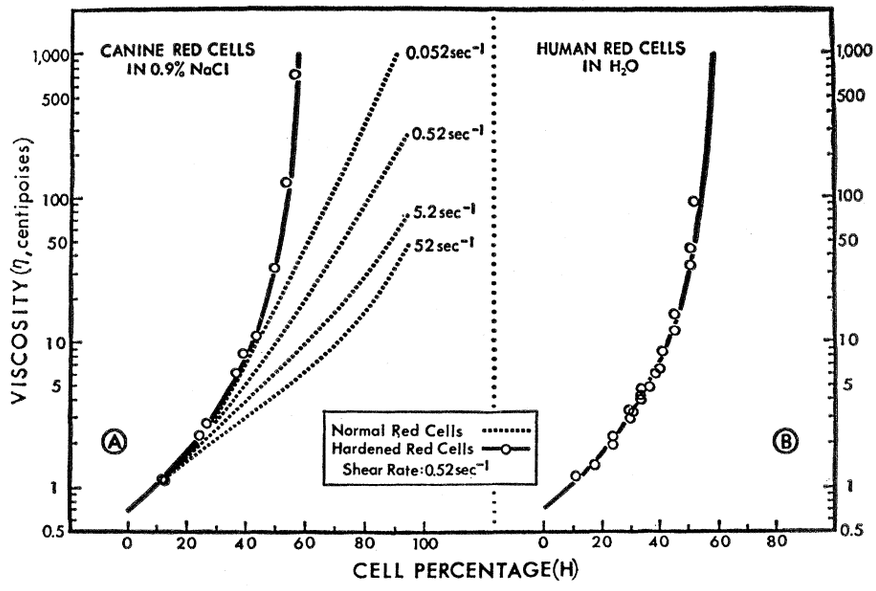

Fig. 2. (A) Relationship between viscosity (log scale) and cell percentage of canine RBC's suspensed in 0.9 percent NaCl. Since hardened RBC's are Newtonian, only the data at a shear rate of 0.52 per second are shown. The results at other shear rates are almost identical. The non-Newtonian behavior of normal RBC's is illustrated by the results obtained at four shear rates. (B) Relationship between viscosity (log scale) and cell percentage of hardened human RBC suspension in water. The data can be fitted by the same curve as the one for results on hardened canine RBC's suspended in 0.9 percent NaCl (Fig. 2A).

cell percentages. The actual cell percentage in each sample was calculated by multiplication of the hematocrit readings (5 minutes at 15,000*g*) by the trapping correction factor (0.60 for hardened RBC's and 0.96 for normal RBC's). The viscosity of the RBC suspensions was determined in a modified version (*6*) of the couette viscometer (*7*) at a temperature of 37°C and shear rates varying from 52 to 0.052 per second.

Figure 1 shows the viscosity-shear rate curves for suspensions of hardened canine RBC's in saline (0.9 percent NaCl). For cell percentages (H) lower than 45 percent, the viscosity is essentially independent of the shear rate with shear rates as low as 0.052 per second; that is, the suspension is nearly Newtonian. As H approaches 60 percent (the upper limit of the packing of hardened RBC's), it becomes more difficult to obtain data at low shear rates, but the suspensions are still essentially Newtonian down to a shear rate of 0.52 per second. Figure 2A shows the relationship between the logarithm of viscosity (η) and H for a shear rate of 0.52 per second (solid line). When H increases from 0 to 30 percent, the viscosity increases only moderately. With H above 40 percent the viscosity rises sharply, and as H approaches 60 percent the viscosity increases toward infinity. The same results were obtained on suspensions of hardened canine RBC's in water and hardened human RBC's in water (Fig. 2B). We have shown that the viscosity of suspensions of rigid polystyrene latex particles (*8*) rises toward infinity in a similar manner when the particle concentration approaches 50 percent.

In contrast to the hardened RBC's, suspensions of normal RBC's can be packed to nearly 100 percent cells, and the viscosity is still within measurable range at such high cell percentages (Fig. 2A). At a given H, the viscosity of the normal RBC suspensions is consistently lower than that of the hardened RBC suspensions. Furthermore, the viscosity of the normal RBC suspensions is clearly dependent on shear rate with H as low as 30 percent. This shear-rate dependence for a normal RBC suspension with H equal to 45 percent is shown in Fig. 3. At very low shear rates (for example, 0.01 per second) the viscosity values are high and close to that of the hardened RBC suspensions. An increase in shear rate causes a progressive reduction in viscosity which then appears to approach a low plateau of approximately 3.6 centipoises with very high shear rates. Studying the viscosity of normal RBC suspensions at high shear rates, Cerny *et al.* (*9*) showed that such a low plateau is reached with shear rates higher than 200 to 300 per second.

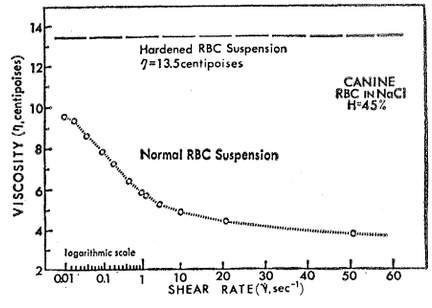

Fig. 3. Relationship between viscosity and shear rate for suspensions of 45 percent canine RBC's in 0.9 percent NaCl. To show clearly the data at low shear rates, a log scale is used for shear rates below 1 per second. Note that the viscosity of normal RBC suspension increases with a reduction in shear rate and that it comes closer to the viscosity of hardened RBC suspension as the shear rate is reduced toward 0. At high shear rates, the viscosity of normal RBC suspension tends to approach a Newtonian region with a low viscosity.

The Newtonian behavior of hardened RBC suspensions and the non-Newtonian property of normal RBC suspensions may be interpreted in the following way. At very low shear rates, when the shear stress acting on the cells is small, normal RBC's are minimally deformed and they have flow properties similar to those of the hardened RBC's. As the shear stress is raised, normal RBC's are gradually deformed, and the suspension viscosity decreases (or the fluidity increases) progressively. On the other hand, the hardened RBC's cannot be deformed, and their suspension viscosity remains constant at a high value, despite the increase of shear stress. With very high shear stresses, the normal RBC's probably become maximally deformed, and their suspensions may enter a Newtonian region with a contant, low viscosity. These viscometric results are in agreement with the findings that normal, deformable RBC's can pass through polycarbonate sieves with 3-μ pores and that hardened RBC's cannot be filtered through even 6.8-μ pores (*2*).

Our experiments were performed on RBC's suspended in protein-free solutions in order to eliminate the complicating factor of cell-protein interactions which exert pronounced effects on blood viscosity (*6*, *10*). In the absence of plasma proteins, the non-Newtonian behavior of the RBC suspension is attributable to the shear deformation of RBC. This deformation factor plays an important role in allowing RBC to flow at very high concentrations when the probability of cell collision (cell-cell interactions) is high. In whole blood with

a normal cell percentage (for example, H = 45 percent), however, the shear deformation of the individual RBC is not as important as the cell-protein interactions (that is, RBC aggregation and shear dispersion) in causing the non-Newtonian behavior (*6*).

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Blood Viscosity: Influence of Erythrocyte Aggregation

Abstract. *The addition of purified canine or bovine fibrinogen to suspensions of canine erythocytes in Ringer solution caused an increase in viscosity and the formation of aggregates of erythocytes. Both of these effects became increasingly pronounced as the fibrinogen concentration was raised, and they approached plateaus with 1 gram of fibrinogen per 100 milliliters. An increase in shear rate (or shear stress) reduced both the effect on viscosity and the aggregate size. The data suggest that fibrinogen causes an increase in blood viscosity and a departure from Newtonian behavior by interacting with erythrocytes to form cell aggregates which can be dispersed by shear stress.*

A suspension of 45 percent erythrocytes (RBC's) in a protein-free salt solution exhibits only slight non-Newtonian characteristics (*1*, *2*), which are attributable to the shear deformation of RBC's (*3*). However, the presence of plasma proteins, especially fibrinogen, results in a marked non-Newtonian behavior (*1*, *2*), presumably caused by the formation of RBC aggregates (*4*) as a result of cell-protein interactions. The addition of a commercial preparation of fibrinogen to saline suspensions of RBC's caused a considerable increase in viscosity at low shear rates (*5*), but the commercial preparation contained a significant amount of proteins other than fibrinogen. We have studied the effects of purified fibrinogen preparations on the viscosity of suspensions of RBC's in Ringer solution and have correlated the effects on viscosity with the tendency of RBC's to aggregate.

Two types of fibrinogen were used. Canine fibrinogen was isolated on the day of the experiments from fresh plasma of normal dogs and was purified by fractional precipitation with ammonium sulfate (*6*). Bovine fibrinogen was prepared by a technique of freezing and thawing (*7*) after the removal of plasma prothrombin by adsorption to barium sulfate (*8*). The bovine preparation was dissolved in 2 percent NaCl (containing 12 mmole of imidazole per liter, *p*H 7.25), stored at −20°C, and thawed at 42° to 45°C on the day of the experiment. By the addition of appropriate salt solutions, the fibrinogen preparations were made to contain the same concentrations of crystalloids as Locke's modification of Ringer solution (*9*). The ability of both types of fibrinogen preparations to clot was higher than 95 percent.

Canine RBC's were washed three times with Ringer solution and then suspended in this solution containing 0 to 1.0 g of fibrinogen per 100 ml. The suspensions were prepared to contain 45 percent RBC's (*10*), and their viscosity was determined in a modified version (*2*) of the couette viscometer (*11*) at shear rates ranging from 52 down to 0.01 per second and at a temperature of 37° ± 0.1°C. Figure 1, A and B, shows the results of the addition of autologous canine fibrinogen and bovine fibrinogen, respectively, to the RBC suspensions, and the effects are closely similar. The addition of either fibrinogen caused a marked elevation of viscosity at low shear rates, greatly enhancing the non-Newtonian behavior of the suspensions. In contrast to fibrinogen, bovine or canine albumin (Mann Research Laboratories, > 95 percent purity) in concentrations up to 4 g per 100 ml caused only a very slight increase in viscosity, and since the increase was nearly equal at all shear rates, the suspension remained essentially Newtonian (Fig. 1B). When both fibrinogen (0.375 g per 100 ml) and albumin (4 g per 100 ml) were present, the viscosity curve of the suspension was only slightly higher than that seen following the addition of fibrinogen alone (Fig. 1B). From these results it can be concluded that the non-Newtonian behavior is caused by fibrinogen but not by albumin within their physiological ranges of concentration and that the effect of fibrinogen is unaffected by the presence or absence of albumin.

Figure 2A summarizes the relation between fibrinogen concentration (ϕ) and suspension viscosity at three selected shear rates. At a given shear rate

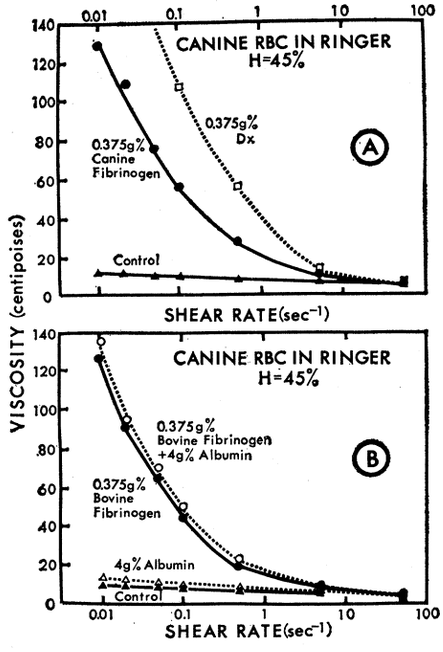

Fig. 1. (A) Effects of canine fibrinogen and dextran (Dx) of high molecular weight on the viscosity of suspensions of 45 percent canine RBC's in Ringer solution at shear rates ranging from 0.01 to 52 per second. (B) Effects of bovine fibrinogen and bovine albumin, individually as well as in combination, on the viscosity of suspensions of 45 percent canine RBC's at shear rates ranging from 0.01 to 52 per second.