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

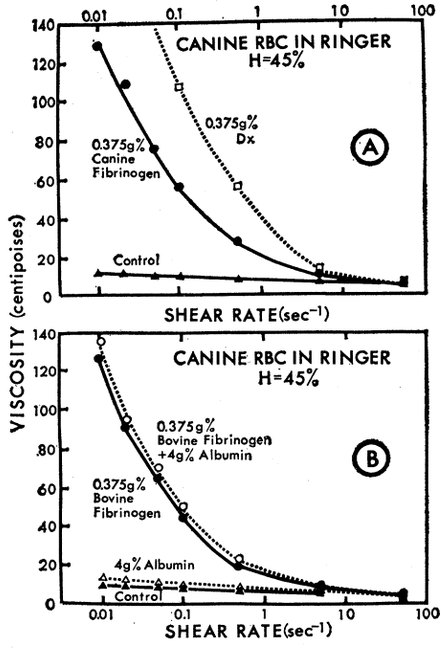

Fig. 1. (A) Effects of canine fibrinogen and dextran (Dx) of high molecular weight on the viscosity of suspensions of 45 percent canine RBC's in Ringer solution at shear rates ranging from 0.01 to 52 per second. (B) Effects of bovine fibrinogen and bovine albumin, individually as well as in combination,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

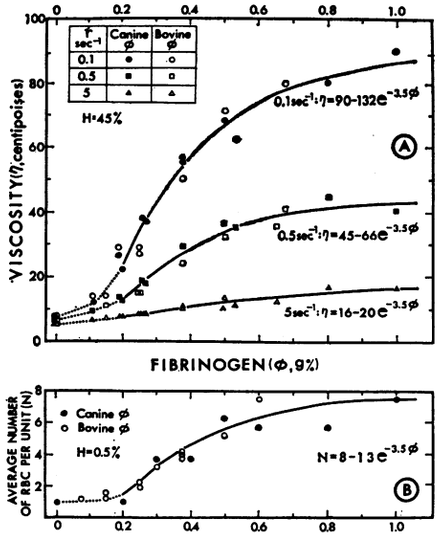

Fig. 2. (A) Relationship between the fibrinogen concentration (ϕ) and the viscosity (η) of 45 percent canine RBC suspensions in Ringer soluton at three shear rates (0.1, 0.5, and 5 per second). (B) Relationship between the fibrinogen concentration and the average aggregate size (N) of 0.5 percent canine or human RBC suspension in Ringer solution. The equations in both panels describe the curves for fibrinogen concentrations higher than 0.2 g/100 ml.

(for example, 0.1 per second), the increase in viscosity was most pronounced when the fibrinogen concentration was elevated from 0.2 to 0.4 g per 100 ml. With an elevation of the fibrinogen concentration to above 0.5 g per 100 ml, the rise in viscosity became less marked and the viscosity appeared to approach a plateau. The viscosity data for fibrinogen concentrations above 0.2 g per 100 ml can be fitted by exponential equations of the form:

$$\eta = \eta_{max} - ae^{-b\phi}$$

The constant b is equal to 3.5 for all three shear rates shown in Fig. 2A, whereas the values for the constants a and η_{max} increase as the shear rate is reduced (*12*). These results indicate that, although the absolute increase in viscosity upon addition of fibrinogen is inversely related to the shear rate, the relative rate of increase in viscosity is independent of shear rates.

We determined the influence of fibrinogen on RBC aggregation by observing microscopically (× 400 magnification) suspensions of 0.5 percent canine or human RBC's in Ringer solution containing 0 to 1.0 g of canine or bovine fibrinogen per 100 ml. Microphotographs were taken of six different fields. In the pictures, each monodispersed RBC or each RBC aggregate was counted as an individual unit. The average number of RBC's per unit was calculated as the ratio of the total number of RBC's (regardless of aggregation) to the number of units. Suspensions of RBC's in Ringer solution without fibrinogen were monodispersed, but the single cells were generally crenated (Fig. 3A). With the addition of a small amount of albumin (for example, 0.25 g per 100 ml) to the Ringer solution, the RBC's assumed their normal biconcave shape (Fig. 3B), but the presence of up to 4 g of albumin per 100 ml did not cause the formation of aggregates. When fibrinogen was added to the Ringer suspension with or without albumin, the RBC's formed aggregates. In the absence of albumin, the aggregates caused by low concentrations of fibrinogen (less than 0.5 g per 100 ml) appeared as clusters of crenated RBC's (Fig. 3C), and rouleaux were found with high concentrations of fibrinogen (more than 0.6 g per 100 ml) (Fig. 3E). When a small amount of albumin (for example, 0.25 g per 100 ml) was added to samples with low concentrations of fibrinogen, the RBC aggregates then also took the form of rouleaux (Fig. 3D), which became tighter as the albumin concentration was raised to 1 g per 100 ml. The RBC aggregation caused by addition of fibrinogen was reversible. The aggregates were dispersed following the application of shear stress by gentle touching of the edge of the cover glass overlying the suspension. After a suspension of RBC's containing fibrinogen was washed in a fibrinogen-free Ringer solution, the previously aggregated RBC's became monodispersed again.

With or without albumin, the size of the aggregates (rouleaux or clusters) increased as the fibrinogen concentration was raised (Fig. 2B). The relation between average aggregate size (N) and fibrinogen concentration (above 0.2 g per 100 ml) can also be described by the exponential equation with the constant b again equal to 3.5 (*12*). Therefore, the effect of increasing fibrinogen concentration on aggregate size (Fig. 2B) is closely similar to its influence on viscosity (Fig. 2A). Furthermore, the effects of fibrinogen on aggregation and viscosity are both affected by the shear rate in a similar manner. At low rates of shear, when RBC aggregates are relatively undisturbed by shear dispersion, the rise in viscosity is most marked. As the shear rate is elevated, the RBC aggregates become dispersed, and the viscosity effect is lessened. At very high rates of shear it is probable that nearly all RBC's are monodispersed despite the presence of fibrinogen, and the viscosity is almost the same as that of a suspension free of fibrinogen (Fig. 1, A and B). The close correlation between the effects of fibrinogen on viscosity and aggregate size strongly suggests that fibrinogen affects the viscosity of RBC suspensions by causing the formation of shear-dependent aggregates. The RBC aggregates are probably formed by the interaction of fibrinogen with the RBC surface, for ex-

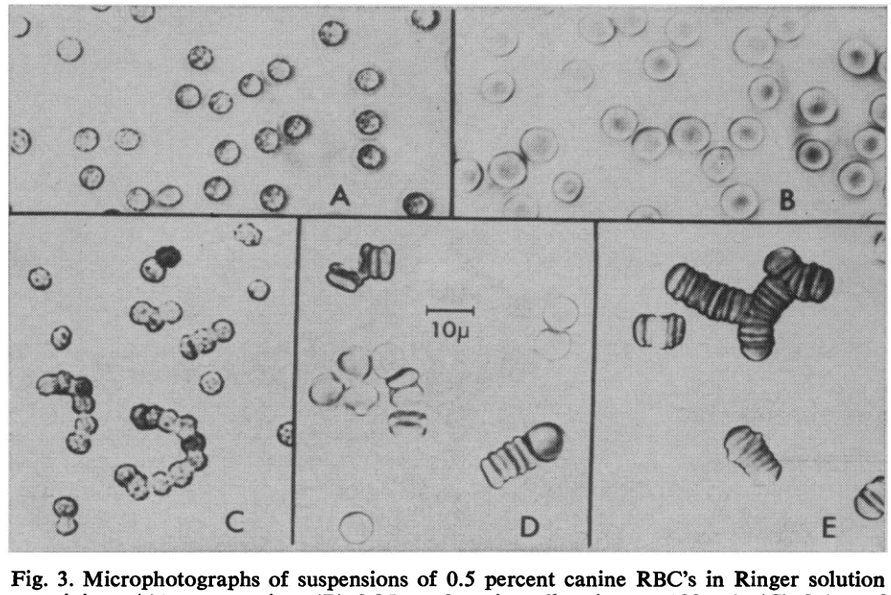

Fig. 3. Microphotographs of suspensions of 0.5 percent canine RBC's in Ringer solution containing: (A) no proteins, (B) 0.25 g of canine albumin per 100 ml, (C) 0.4 g of canine fibrinogen per 100 ml, (D) 0.4 g of canine fibrinogen and 0.25 g of canine albumin per 100 ml, and (E) 0.8 g of canine fibrinogen per 100 ml.

ample, adsorption of fibrinogen (*13*).

Although there is good correlation between the effects of fibrinogen on blood viscosity and RBC aggregation, it must be noted that these two effects were studied under different experimental conditions. The aggregate size was estimated at zero shear rate, whereas the viscosity was measured at shear rates approaching, but not equal to, zero. Because of technical limitations, microscopic observations on RBC aggregation were made on suspensions containing 0.5 rather than 45 percent RBC's. It is clearly desirable to obtain data on RBC aggregation at cell concentrations and shear rates comparable to those in viscometry.

The experiments on fibrinogen (molecular weight 340,000) have been repeated on a dextran with a high molecular weight (Dx 375, mean molecular weight 375,000, Pharamacia Corp., Uppsala, Sweden). The Dx 375 caused an increase of the viscosity of RBC suspensions preferentially at low shear rates (Fig. 1A) as well as the formation of RBC aggregates. These effects were much less pronounced when dextrans of lower molecular weight (for example, 72,000) were used (*14*). Therefore the marked effects on viscosity and aggregate size observed with fibrinogen but not with albumin are probably related to the larger molecular size of fibrinogen.

We conclude that purified autologous or heterologous fibrinogen causes RBC aggregation and a rise in viscosity preferentially at low shear rates. The shear dispersion or deformation of the RBC aggregates formed by cell-protein interactions is a major mechanism responsible for the non-Newtonian behavior of whole blood at normal cell percentages (*2*). Another factor leading to the non-Newtonian behavior of whole blood is the shear deformation of the individual RBC's (*3*), and such direct cell-cell interactions become important when the cell percentage is elevated above normal (*2*).

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9. This solution contains 0.9 g of NaCl, 0.042 g of KCl, 0.02 g of $NaHCO_3$, 0.024 g of $CaCl_2$, 0.1 g of glucose, and 20 mg of heparin per 100 ml. The *p*H is adjusted to 7.2.
10. The cell percentage was adjusted to 45 percent before viscometry, but was often found to increase by approximately 4 percent after viscometry in the samples containing fibrinogen. A correction for this change in cell percentage would lower the viscosity values slightly but would not alter the general shape of the viscosity curves.
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12. As the shear rate is reduced, the increases in "*a*" and η_{max} are almost parallel to each other and the ratio a/η_{max} is fairly constant (1.3 to 1.5 for the three shear rates shown in Fig. 2A). This ratio is also close to the corresponding ratio calculated for relation of aggregate size to fibrinogen concentration, where a/N_{max} is equal to 1.6.
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Athens Variant of Glucose-6-Phosphate Dehydrogenase

Abstract. *A variant of glucose-6-phosphate dehydrogenase (G6PD), characterized by slower than normal electrophoretic migration and associated with mild deficiency of G6PD in the red cells, was detected in two unrelated Greek males. Electrophoretic, chromatographic, and enzymologic study indicated that the new mutant is structurally different from normal G6PD (B+) and from the Mediterranean variant associated with red-cell enzyme deficiency (B−). Convincing electrophoretic separation of the new variant from the normal B+ and the Mediterranean B− enzymes was achieved only by detailed electrophoretic study in different buffer systems and conditions.*

The common type of deficiency of glucose-6-phosphate dehydrogenase (G6PD) observed in Caucasians is always associated with almost absent or very low enzyme activity in red cells (from 0 to 5 percent of normal); in electrophoretic mobility the enzyme is indistinguishable from normal B+ G6PD. This mutant, mainly found in populations of Mediterranean origin, is usually called the Mediterranean (or B−) variant of G6PD (*1*). In Greeks another variety of G6PD deficiency, associated with erythrocyte G6PD activities from 10 to 50 percent of normal, is also observed and usually referred to as the mild Greek type of G6PD deficiency (*2*). A number of Greek males having this mild type of G6PD deficiency had been studied by electrophoresis by use of either the tris-HCl (*3*) or the tris-EDTA-borate (EDTA, ethylenediaminetetraacetic acid) (*4*) buffer system; mainly they had erythrocyte G6PD migrating with the same mobility as the normal B+ enzyme (*5*). Recently the G6PD from two unrelated males, having mild deficiency of G6PD and supposedly normal electrophoretic mobility, was subjected to more detailed electrophoretic and enzymologic study. In both instances an identical, previously undescribed, G6PD mutant, differing in electrophoretic and enzymologic characteristics from normal (B+) and Mediterranean (B−) enzyme, was detected. The new mutant was not associated with hemolytic anemia. Since the first case examined originated from Athens (Greece), the new G6PD variant will be referred to as G6PD Athens.

On starch-gel electrophoresis, using a tris-EDTA-borate buffer system and the routine electrophoretic conditions described in the legend to Fig. 1, G6PD Athens migrates on the tail of the normal B+ G6PD. In a comparison of the electrophoretic behaviors of G6PD's Seattle (*6*), Athens, normal B+, and Negro A+, their relative mobilities were 92, 98, 100, and 108 percent (Fig. 1). Convincing separation of G6PD Athens from the normal B+ was achieved by high-voltage electrophoresis (Fig. 2) and by electrophoresis on phosphate buffer, *p*H 7.0 (Fig. 3). In another experiment in which starch-gel electrophoresis of G6PD's A+, B+, Athens, and Seattle was carried out in phosphate buffer, *p*H 7.0, for 20 hours at 4 volt/cm, the distances of the four dehydrogenases from the application point were: A+, 6.7 cm; B+, 5.7 cm; Athens, 4.6 cm; and Seattle, 3.9 cm.