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Research Article

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ABSTRACT The viscosity of *oxygenated* blood from patients with sickle cell anemia (Hb SS disease) was found to be abnormally increased, a property which contrasts with the well recognized viscous aberration produced by deoxygenation of Hb SS blood. Experiments designed to explain this finding led to considerations of deformation and aggregation, primary determinants of the rheologic behavior of erythrocytes as they traverse the microcirculation. Deformability of erythrocytes is in turn dependent upon internal viscosity (i.e. the state and concentration of hemoglobin in solution) and membrane flexibility. Definition of the contribution made by each of these properties to the abnormal viscosity of oxygenated Hb SS blood was made possible by analysis of viscosity measurements, made over a wide range of shear rates and cell concentrations, on Hb SS erythrocytes and normal erythrocytes suspended in Ringer's solution (where aggregation does not occur) and in plasma. Similar measurements were made on the two cell types separated by ultracentrifugation of Hb SS erythrocytes: high density erythrocytes composed of 50 to 70% irreversibly "sickled" cells (ISC) and low density erythrocytes composed of over 95% non-ISC.

Under all experimental conditions (hematocrit, shear rate, and suspending medium) the viscosity of ISC exceeds that of normal erythrocytes. The viscosity of non-ISC is elevated only in the absence of aggregation and over intermediate ranges of hematocrit. Analyses of the data reveal (*a*) an elevated internal viscosity of ISC; (*b*) a reduced membrane flexibility of both ISC and non-ISC, particularly at low shear rates; and (*c*) a reduced tendency for aggregation displayed by both cell types.

The abnormal viscosity of oxygenated Hb SS blood can be attributed to the altered rheology of ISC and, to a lesser extent, of non-ISC. These studies assign a role to the abnormal rheology of Hb SS erythrocytes in the pathogenesis of sickle cell anemia, even under conditions of complete oxygenation.

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INTRODUCTION

The possible significance of increased blood viscosity in sickle cell anemia (Hb SS disease) under conditions associated with deoxygenation of erythrocytes has long been recognized (1). Presumably the phenomenon of reversible sickling reflects a conformation of erythrocyte membranes to bundles of parallel filaments composed of Hb S molecules in the deoxygenated state (2–6). These cells are, in turn, responsible for the abnormal rheology of deoxygenated Hb SS blood, a property which can be demonstrated in various ways. Measurements in a capillary viscometer (7) showed that the viscosity of Hb SS blood adjusted to a packed cell volume (hematocrit) of 35% begins to increase when oxygen tension is reduced to 60 mm Hg, and that on further deoxygenation the viscosity ultimately exceeds that of oxygenated Hb SS blood by a factor of 3 to 4. The viscosity of packed Hb SS erythrocytes measured between coaxial cones in a rotational viscometer increased 10- to 30-fold upon deoxygenation (8). It has been suggested that viscous impedance to blood flow through small vessels leads to further lowering of oxygen tension, and a self-perpetuating cycle of augmented sickling and increasing viscosity develops (1).

In contrast to erythrocytes which remain sickled only when deoxygenation is maintained, other erythrocytes in Hb SS blood retain their sickled shape even after oxygenation (9). These "irreversibly sickled cells" (ISC) may form as a consequence of in vivo sequestra-

TABLE I

Routine Hematological Data on Hb SS Patients

Patient	Sex	Age	Hematocrit	ISC	Hb	Hb F
			%	%	(*g/100 ml*)	%
M. C.	F	18	20.5	22	7.1	2.3
L. H.	M	8	23.4	15	7.4	11.0
C. P.	M	11	22.0	22	7.8	8.8
R. S.	F	13	23.0	21	8.4	11.7
B. W.	F	24	18.0	24	6.0	2.8

tion and an ensuing prolonged hypoxia (10), a mechanism analogous to the irreversible sickling of erythrocytes produced by anaerobic incubation of Hb SS blood in vitro (11). Once formed, ISC suffer preferential destruction (12).

Whereas previous rheological investigations of Hb SS blood have emphasized the role of deoxygenation, the substance of this report is (*a*) the finding of an increased viscosity of oxygenated Hb SS blood, and (*b*) a probable explanation of this increased viscosity in terms of the membrane flexibility and hemoglobin content of ISC and non-ISC.

METHODS

Patients. Blood was obtained from patients with proven Hb SS disease. None was in sickle cell crisis at the time of the study, and none had been transfused in the preceding 4 months. All measurements were performed within 8 hr of drawing venous blood into ethylenediaminetetraacetate (EDTA, 1 mg/ml blood).

Separation of ISC from non-ISC by ultracentrifugation. Venous blood, oxygenated by equilibration with 95% O_2—5% CO_2 and with hematocrit adjusted to approximately 60% was centrifuged at 20°C in a Spinco SW 50 L swinging bucket rotor[1] for 1 hr at 40,000 rpm (12). The upper layer of cells, mainly reticulocytes, was discarded; and the remaining cell column was divided into an ISC-poor top fraction and an ISC-rich bottom fraction (12).

Estimation of per cent of irreversibly sickled cells. Two matched cover glass films of blood previously equilibrated with 95% O_2–5% CO_2 were stained with Wright's stain and 200 cells on each were classified as ISC or non-ISC for each determination (12). The precision of this subjective assay was approximately ±5% (SD).

Calculation of RBC indices. Packed cell volumes were read in capillary tubes after centrifugation for 5 min at 15,000 *g*. True hematocrit (H) was calculated from the packed cell volume with the use of a plasma trapping correction factor (13) determined separately for the top and bottom fractions with the use of albumin-^{131}I. In the text to follow, "hematocrit" signifies true hematocrit corrected for trapping.

Red cell counts (RBC) were determined in a Coulter electronic counter (model B, Coulter Electronics, Hialeah, Fla.). Hemoglobin concentration (Hb) was measured as cyanmethemoglobin (14). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated from H, RBC, and Hb. Per cent Hb F was estimated by the 1 min technique of Singer, Chernoff, and Singer (15).

Total serum protein concentration was measured by a refractometric method (16) calibrated by micro-Kjeldahl determinations (17). Serum protein fractions were assayed by electrophoresis on cellulose acetate membranes and by subsequent scanning in an Analytrol densitometer (Beckman Instruments). Plasma fibrinogen concentration was calculated from tyrosin-like activity in the fibrin clot (18).

Viscometry. Viscosity measurements were made at 37°C on suspensions of top fraction erythrocytes (poor in ISC), bottom fraction erythrocytes (rich in ISC), and original mixtures of ISC and non-ISC prior to ultracentrifugation. A coaxial cylinder viscometer (19) was used. The two cylinders are separated by an annular gap of 960 μ containing the sample. A guard ring is present at the air-sample interface to prevent the formation of surface films. The inner cylinder is rotated at a constant speed; the outer cylinder, which rides on an air bearing, is held stationary by the torque generated from an electronic feedback system. Shear rate, calculated from rotation speed and cylinder geometry, can be varied from 52 to 0.01 sec^{-1}. Shear stress is calculated from generated torque using standard viscosity oils. Apparent viscosity (η, hereafter referred to as viscosity) is the ratio of shear stress to shear rate.

For each patient, separate viscometry studies were made at a series of different hematocrits on (*a*) erythrocytes suspended in autologous plasma, and (*b*) erythrocytes washed and suspended in Ringer's solution containing 0.25% human serum albumin and buffered to pH 7.4 (5 mM phosphate or 12 mM Tris). All viscosity measurements were performed on samples immediately after equilibration with 95% O_2–5% CO_2 (suspensions in plasma) or room air (suspensions in Ringer's solution).

Viscosity measurements were also made on membrane-free hemolysates of ISC, non-ISC, and normal erythrocytes prepared by a technique described by Cokelet and Meiselman (20).

RESULTS

Hematological data. Routine hematological data on original blood samples are shown in Table I. ISC counts varied from 15 to 24%. After ultracentrifugation of the blood samples, top fractions contained less than 3% ISC, whereas ISC were concentrated to between 50 and 70% in bottom fractions (Table II). For purposes of discussion, therefore, top fraction erythrocytes will be referred to as non-ISC and bottom fraction erythro-

[1] Beckman Instruments Inc., Spinco Division, Palo Alto, Calif.

TABLE II

Hematological Parameters of Top and Bottom Fractions of Hb SS Blood

Hb SS Patients	MCV		MCH		MCHC		ISC	
	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom
	μ^3		$\gamma\gamma$		*g/100 ml*		%	
M. C.	85.3	64.0	27.8	27.2	32.7	40.8	0.5	51
L. H.	86.8	69.3	27.2	27.0	31.4	39.0	2.5	50
C. P.	84.4	68.1	28.1	29.2	33.6	42.9	2.5	70
R. S.	96.5	74.9	29.2	30.0	30.2	40.2	2.5	52
B. W.	105.8	78.2	34.7	34.1	31.6	43.1	1.0	61
Mean	91.8	70.9*	29.4	29.5	31.9	41.2*	1.8	57
SD	9.2	5.6	3.0	2.9	1.3	1.8	1.0	9
Normal								
Mean	90.3		30.1		33.5		0	
SD (n)	6.3 (15)		2.3 (6)		2.6 (6)			

* t tests on difference between bottom and top fractions as well as that between bottom fraction and normal blood show $P < 0.01$.

cytes as ISC. In agreement with previous studies (12) the MCV of ISC was consistently smaller than that of non-ISC, but the two were identical in MCH: hence the MCHC of ISC exceeded that of non-ISC. The trapping correction factor averaged 0.94 (SD 0.02, n = 8) for ISC and 0.96 (SD 0.02, n = 7) for non-ISC: the difference was not statistically significant ($0.1 > P > 0.05$). Compared to the trapping correction factor of 0.99 in normal blood (13), these values for ISC and non-ISC are significantly lower ($P < 0.01$ for ISC and $P < 0.05$ for non-ISC). Hb SS patients had higher than normal values for plasma viscosity and plasma protein concentration (Table III). The higher plasma protein concentration resulted primarily from elevated gamma globulin levels, a finding in agreement with results obtained with paper electrophoresis (21, 22).

Effect of shear rate on viscosity of Hb SS whole blood. Fig. 1 shows the relationship between viscosity and shear rate for oxygenated Hb SS blood and normal blood with hematocrit adjusted to 45%. Both exhibited a shear thinning behavior, i.e., an inverse relationship between viscosity and shear rate. At any shear rate, the viscosity of Hb SS blood was significantly higher than that of normal blood ($P < 0.01$).

After appropriate cross-matching of cells and plasma

TABLE III

Plasma Viscosity and Protein Concentration in Hb SS Patients

Patient	Plasma viscosity	Protein concentration							
		Total	Alb.	α_1	α_2	β_1	β_2	γ	ϕ
	centipoises	*g/100 ml*							
M. C.	1.48	8.98	3.71	0.17	0.47	0.90	1.04	2.34	0.35
L. H.	1.20	7.24	4.50	0.26	0.57	0.41	0.42	0.88	0.20
C. P.	1.39	8.05	4.66	0.19	0.39	0.58	0.57	1.36	0.30
R. S.	1.25	7.61	4.54	0.15	0.53	0.71	0.39	1.12	0.25
B. W.	1.41	8.32	4.51	0.26	0.78	0.61	0.29	1.56	0.32
Mean	1.35*	8.04‡	4.37	0.21	0.55	0.64	0.54	1.45‡	0.28
SD	0.11	0.67	0.39	0.05	0.15	0.18	0.30	0.56	0.06
Normal (n = 6)									
Mean	1.18	7.40	4.69	0.23	0.48	0.53	0.33	0.88	0.27
SD	0.07	0.50	0.44	0.02	0.15	0.19	0.27	0.19	0.04

* t test on difference between Hb SS and normal values shows $P < 0.01$.

‡ t test on difference between Hb SS and normal values shows $P < 0.05$.

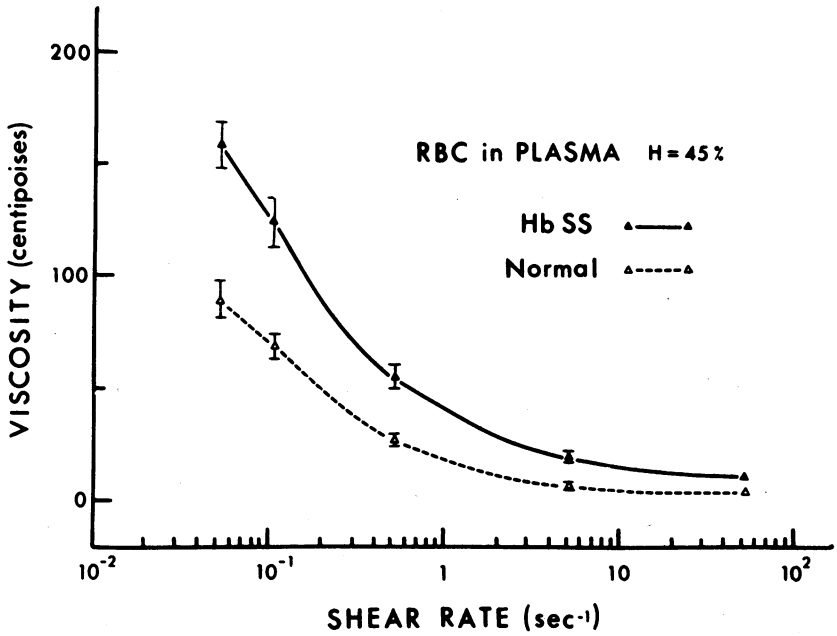

FIGURE 1 Relationship between viscosity (linear scale) and shear rate (log scale) for oxygenated Hb SS blood (n = 5) and normal blood (n = 6) at 45% hematocrit. Values shown are means ±SEM.

from an Hb SS patient and from a normal subject, erythrocytes from each were washed with and suspended in the plasma of the other. As Fig. 2 shows, erythrocytes are responsible for at least 2/3 of the difference in viscosity between Hb SS and normal blood, and for almost the entire difference at lowest shear rates.

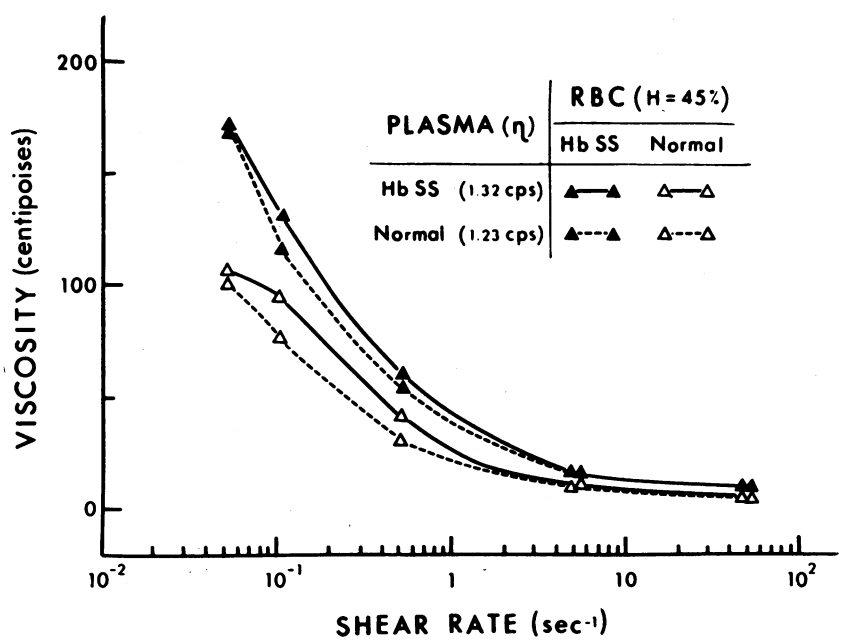

FIGURE 2 Relationship between viscosity and shear rate for suspensions of oxygenated Hb SS and normal erythrocytes in autologous plasma and in cross-exchanged plasma. Hematocrit equal 45% in all suspensions. The viscosity of Hb SS erythrocytes exceeded that of normal erythrocytes in either plasma.

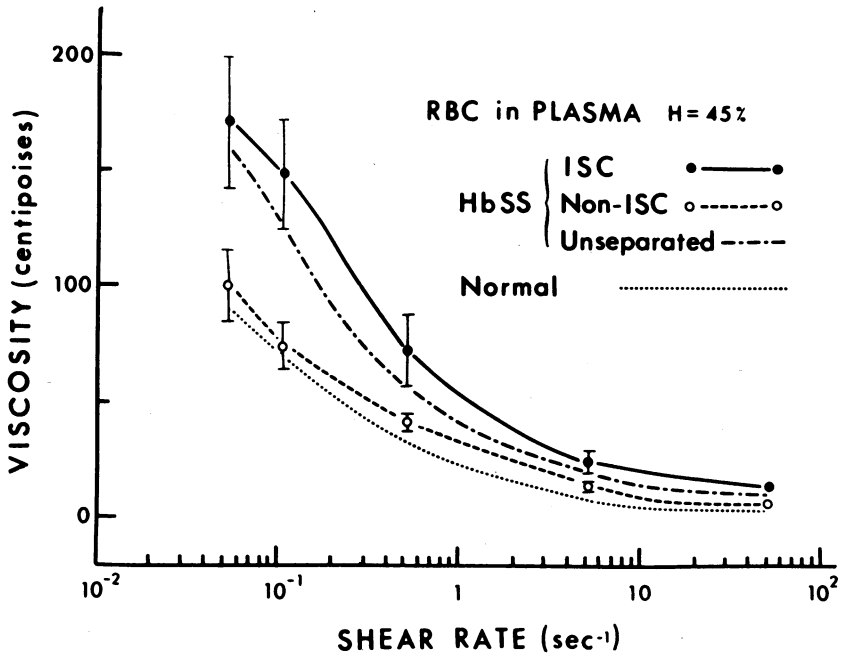

FIGURE 3 Relationship between viscosity and shear rate for suspensions of oxygenated ISC and non-ISC in plasma. Values shown are means ±SEM. Also shown for comparison are data on unseparated Hb SS blood and normal blood. Hematocrit equals 45% in all suspensions.

Effect of shear rate on viscosity of suspensions of ISC and non-ISC in plasma. At an hematocrit of 45%, suspensions of ISC in plasma had viscosity values almost twice as high as those for non-ISC, with unseparated Hb SS erythrocytes being intermediate (Fig. 3). Fig. 4 shows the viscosity-shear rate relationship (log-log plot) for suspensions of ISC and non-ISC in plasma at three hematocrit levels. The difference in viscosity between these two cell types was significant at each hematocrit level, but it was more pronounced at high hematocrits.

Effect of suspending medium on comparative viscosity of Hb SS and normal erythrocytes. Suspensions of Hb SS and normal erythrocytes in Ringer's solution at 45% hematocrit showed lower viscosity values than their suspensions in plasma (Fig. 5), and the divergence was greatest at low shear rates. Suspended in Ringer's solution, Hb SS erythrocytes showed viscosity values approximately twice those of normal erythrocytes.

Relationship between viscosity and hematocrit: ISC and non-ISC suspended in plasma. The influence of hematocrit on the viscosity of ISC and non-ISC is shown by the composite plot (all five patients) of the data at two selected shear rates (0.052 and 52 sec^{-1}) together with the range of normal (Fig. 6 A). Non-ISC at all hematocrits showed essentially the same viscosity at 0.052 sec^{-1} as normal blood. At 52 sec^{-1}, the viscosity of non-ISC diverged significantly from normal blood at intermediate hematocrits, but the difference vanished when the hematocrit was raised to 80% or above.

At 45% hematocrits and above, the viscosity of ISC was significantly higher than that of either normal blood or non-ISC ($P < 0.01$). The difference in viscosity between ISC and non-ISC at both shear rates became greater at high hematocrits: approximately 2-fold at 45% hematocrit, and 5-fold at 90% hematocrit (Fig. 6 A).

Relationship between viscosity and hematocrit: ISC and non-ISC suspended in Ringer's solution. The viscosity of non-ISC diverged significantly from normal at intermediate hematocrit range, both at high and low shear rates (Fig. 6 B). This behavior is the same as that noted for suspensions of non-ISC in plasma at high shear rate (Fig. 6 A).

The viscosity of ISC in Ringer's solution was higher than that of normal erythrocytes at all hematocrits and was higher than that of non-ISC at hematocrits above 45% (Fig. 6 B).

The data of Fig. 6 are replotted in Fig. 7 to permit a more ready comparison of the relative viscosity of a given cell type (ISC or non-ISC) in two different suspending media (plasma or Ringer's solution). For both ISC (Fig. 7 A) and non-ISC (Fig. 7 B) the relation between relative viscosity and hematocrit at 52 sec^{-1} was essentially independent of the suspending medium. At the lower shear rate (0.052 sec^{-1}), the viscosity curves

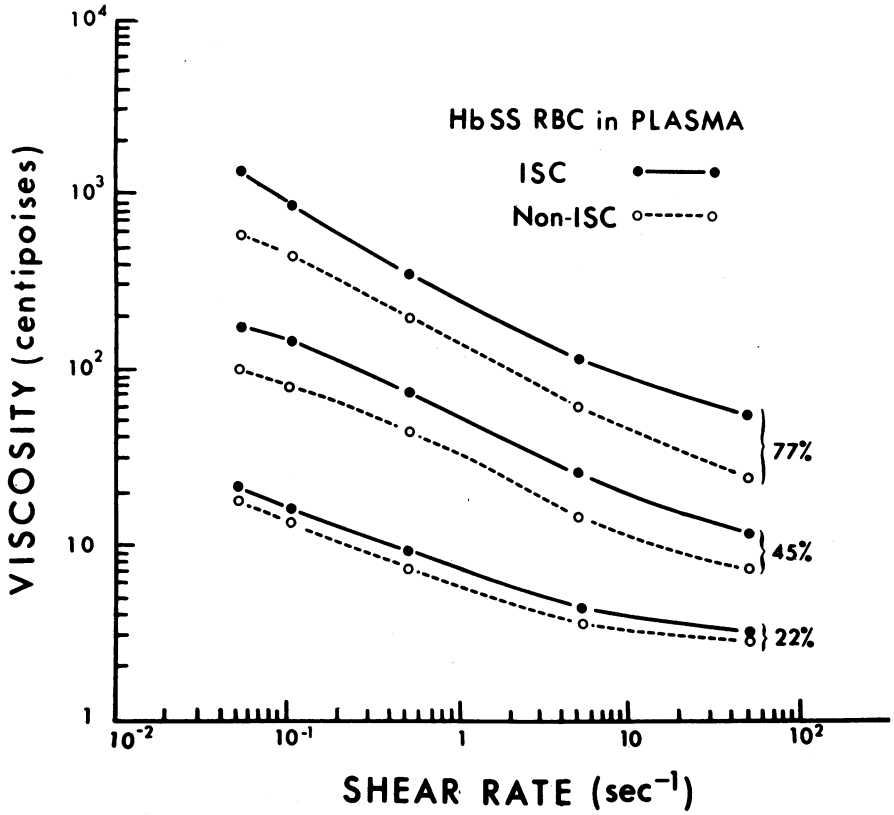

FIGURE 4 Relationship between viscosity and shear rate (log-log plot) for suspensions of oxygenated ISC and non-ISC in plasma at three hematocrit levels (77, 45, and 22%). Points plotted are means of five patients.

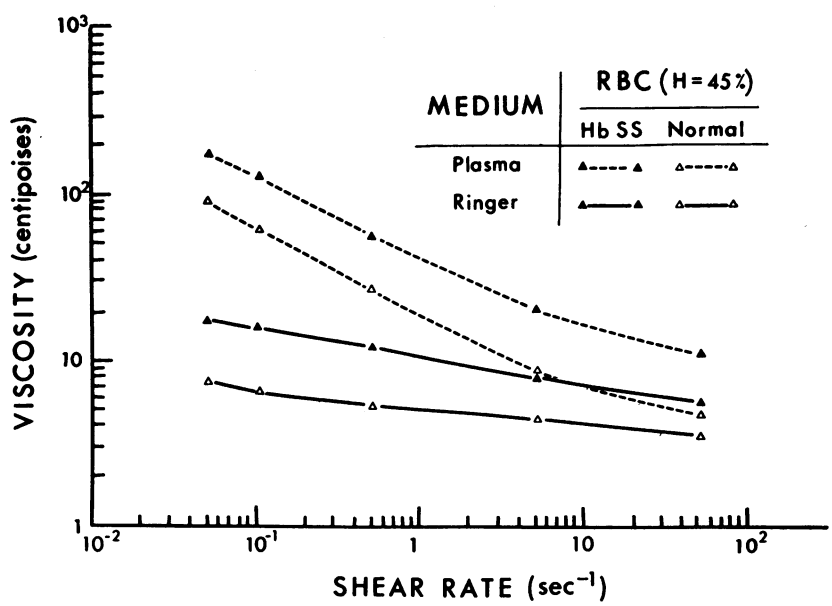

FIGURE 5 Relationship between viscosity and shear rate for suspensions of oxygenated Hb SS (unseparated) and normal erythrocytes in Ringer's solution and in plasma at an hematocrit of 45%.

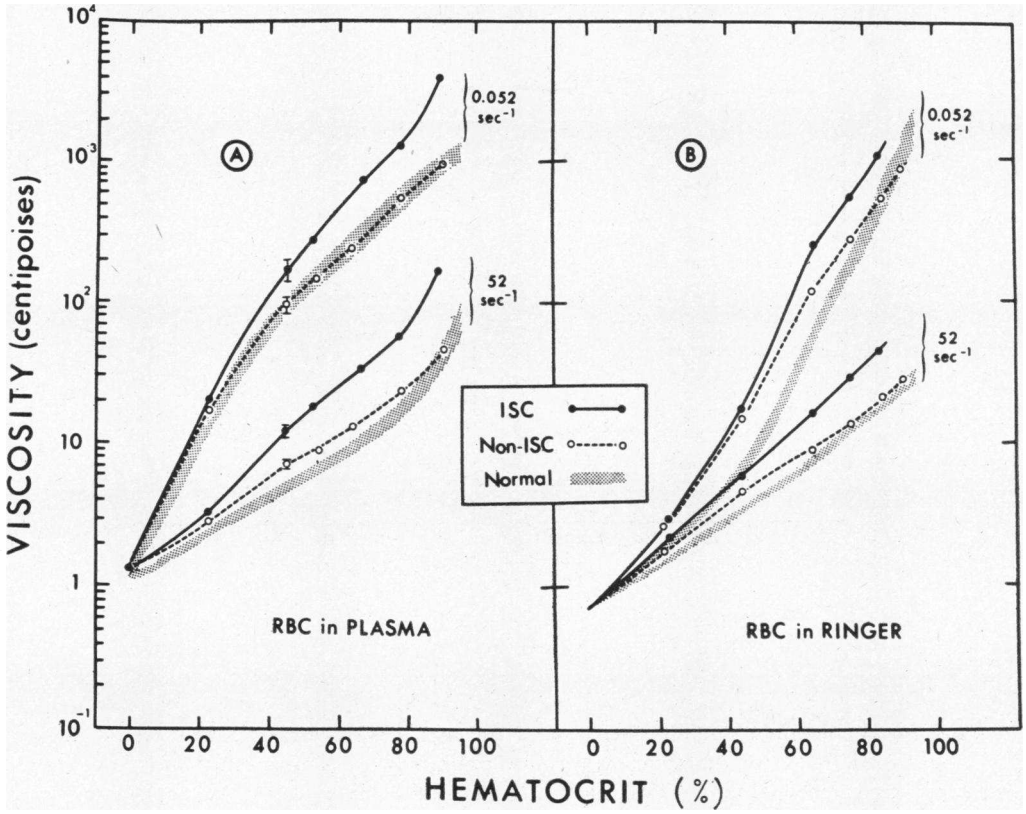

FIGURE 6 Relationship between viscosity and hematocrit for suspensions of oxygenated ISC, and non-ISC, and normal erythrocytes in plasma (A) and in Ringer's solution (B). Data on two shear rates (0.052 and 52 sec^{-1}) are given. Values for ISC and non-ISC are means; a representative SEM is shown at 45% hematocrit in A. The shaded areas include the entire range of data obtained on six normal subjects.

for both cell types showed a marked dependence on suspending media with sharp divergence at intermediate hematocrits. This dependence on suspending media vanished at higher hematocrits.

Viscosity of hemolysates. The viscosities of membrane-free hemolysates prepared from ISC, non-ISC, and normal erythrocytes all varied similarly with the hemoglobin concentration (Fig. 8). The data agree with those obtained by others on Hb AA (20, 23–25) as well as on Hb SA hemolysates (25). An increase in hemoglobin concentration up to the normal MCHC (32 g/100 ml) caused a relatively slight rise in viscosity, but further elevations in hemoglobin concentration resulted in a sharp increase in viscosity (Fig. 8).

DISCUSSION

A major finding evolving from these studies is that, even under conditions of complete oxygenation, the viscosity of Hb SS blood exceeds that of normal blood at the same hematocrit (Fig. 1). In previous studies on blood viscosity in Hb SS disease (e.g. references 1, 7, 26), attention was centered on changes occurring after deoxygenation, and the only recording of an abnormal viscosity of oxygenated Hb SS blood was made on packed erythrocytes (8). Our studies further reveal a rheological difference between the two morphologically distinct types of oxygenated Hb SS erythrocytes: non-ISC and ISC.

Recent studies have shown that erythrocyte aggregation (27) and erythrocyte deformation (28) are major factors determining blood viscosity. The deformation of erythrocytes at high shear stress involves membrane bending (29) which in turn permits changes in cell shape and an alignment of cell axis with flow (30, 31). Furthermore, the flexible membrane can transmit shear stress to the interior fluid, which then undergoes a circular motion to participate in flow (31). Therefore deformability of erythrocytes, a property which results in a lowering of viscosity at high shear rates (28), de-

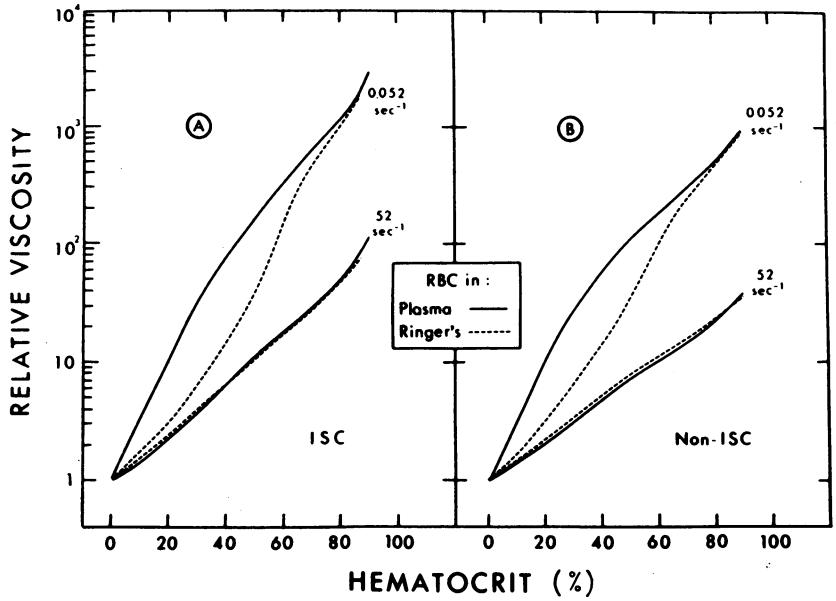

FIGURE 7 Relationship between relative viscosity and hematocrit for suspensions of oxygenated ISC (A) and non-ISC (B) in plasma and Ringer's solution.

pends on the internal viscosity [2] of the hemoglobin-rich solution as well as on the flexibility of the membrane [3] (20, 29, 31, 32). An explanation of the abnormally increased viscosity of oxygenated Hb SS blood necessitates individual considerations of these rheological factors: membrane flexibility, internal viscosity, and erythrocyte aggregation. In the discussion to follow, suspension systems are chosen to permit consideration of the three factors in the order given.

Membrane flexibility of Hb SS erythrocytes: comparison of non-ISC and normal erythrocytes in Ringer's solution. To permit isolated assessment of the role of membrane flexibility, comparisons ideally should be sought between suspensions of nonaggregating erythrocytes with identical internal viscosities. Aggregation does not occur in suspensions of erythrocytes in Ringer's solution (27). Non-ISC and normal erythrocytes have comparable values of MCHC (Table II) and, according to the data shown in Fig. 8, presumably possess similar internal viscosities. When suspended in Ringer's solution, however, non-ISC have a higher viscosity than normal over an hematocrit range of 20–65% (Fig. 6 B). The logical interpretation of this behavior of non-ISC is that their membranes are less flexible than normal. Since the difference in viscosity between non-ISC and normal erythrocytes was least at the higher shear rate (Fig. 6 B), it is apparent that at high shear rates membrane flexibility of non-ISC approaches that of normal erythrocytes and that the membrane abnormality of non-ISC manifests itself mainly at low shear rates. This interpretation of the increased viscosity of non-ISC at low shear rates as a manifestation of lessened membrane flexibility is supported by studies on erythrocytes partially hardened with acetaldehyde (33). The pathophysiological implication of such shear-dependent alteration of membrane flexibility in non-ISC is as follows. At high rates of blood flow (high shear rates), non-ISC membranes behave essenially as membranes of normal erythrocytes. However when there is a reduction in blood flow (low shear rates) the decrease of membrane flexibility in non-ISC may cause an elevation of blood viscosity and impede passage of these cells through the microcirculation.

The demonstration of ISC membrane damage in transmission electron microscopy (34), the probable attrition of their membrane material during sickle-unsickle cycles (35), and the finding that ISC retain the same sickled shape after hypotonic lysis (36) all strongly suggest that ISC as well as non-ISC have a lower membrane flexibility than normal erythrocytes.

Internal viscosity of Hb SS erythrocytes: comparison of ISC and non-ISC in Ringer's solution. As a result of the marked difference in MCHC (Table II) between ISC (41 g/100 ml) and non-ISC (32 g/100 ml) the internal viscosity of ISC should be correspondingly

[2] The term "internal viscosity" is used here to denote the viscosity of the internal fluid surrounded by erythrocyte membrane. Therefore it is a function of the state and concentration of hemoglobin in this fluid and, for present purposes, not of membrane flexibility.

[3] Membrane flexibility is a function of the tensile properties of the membrane and of the relation between cell volume and surface area.

higher. The higher viscosity values for ISC in Ringer's solution than those for non-ISC in Ringer's solution (Fig. 6 B) are at least partly explicable on this basis. The contribution of membrane flexibility to this viscosity difference between ISC and non-ISC cannot be precisely assessed. The association of a high MCHC with an elevated blood viscosity has been shown in two other hematological disorders involving aberrant erythrocytes: hereditary spherocytosis (37) and homozygous Hb C diseases (23, 38). A similar correlation exists in suspensions of erythrocytes obtained under a variety of conditions: high pH (39), hypertonic shrinkage (40), and erythrocyte aging (41).

Aggregation of Hb SS erythrocytes: comparison of Hb SS and normal erythrocytes in plasma. In the presence of plasma proteins, particularly fibrinogen and several globulin fractions, normal erythrocytes aggregate to form rouleaux which can be dispersed by shearing (27, 42). Therefore, decreases in shear cause an increase in viscosity which is greater for suspensions in plasma than for suspensions in Ringer's solution (43). Although Hb SS erythrocytes sickled by deoxygenation do not aggregate (44), the oxygenated ISC and non-ISC in the present study were consistently observed to form aggregates in the presence of plasma proteins. At low shear rates (e.g. 0.052 sec^{-1}), the viscosity differences between suspensions in plasma and suspensions in Ringer's solution of either ISC (Fig. 7 A) or non-ISC (Fig. 7 B) necessarily reflect the formation of cell aggregates in plasma. The disappearance of the difference at 90% hematocrit (Fig. 7) in turn can be explained by the dominant influence of cell crowding over effects of cell aggregation (19). At high shear rates (e.g. 52 sec^{-1}), when the aggregates are nearly completely dispersed by shear, suspensions of Hb SS erythrocyte fractions in plasma show essentially the same viscosity as their corresponding suspensions in Ringer's solution (Fig. 7). This relative independence of rheological behavior of erythrocyte suspensions on the nature of suspending media at high hematocrits or high shear rates is a general phenomenon previously recognized with normal cells suspended in different systems (19).

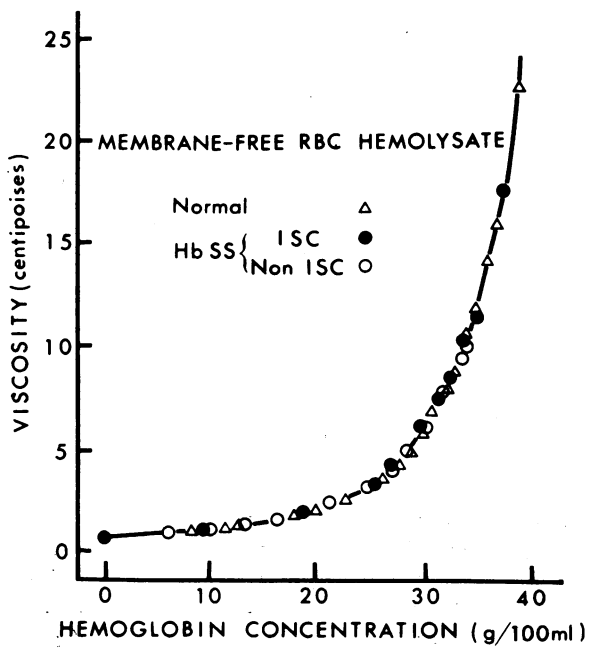

FIGURE 8 Relationship between viscosity and hemoglobin concentration of membrane-free hemolysates prepared from oxygenated ISC, non-ISC, and normal erythrocytes.

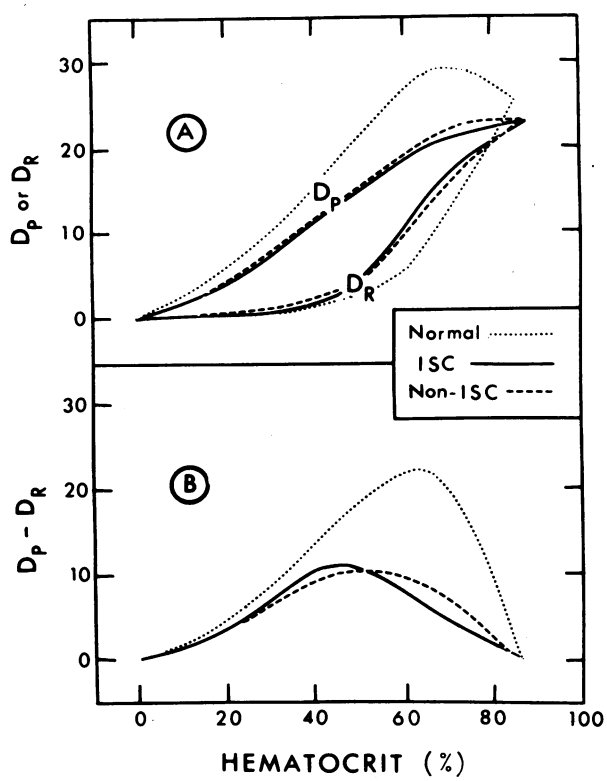

FIGURE 9 A. Shear dependence of viscosity of suspensions of oxygenated ISC, non-ISC, and normal erythrocytes in plasma (D_P) and in Ringer's solution (D_R) as functions of hematocrit. B. Relationship between ($D_P - D_R$) and hematocrit. At an hematocrit of 45% the value of ($D_P - D_R$) is approximately 50% higher for normal erythrocytes than for ISC and non-ISC.

The viscosity of both normal erythrocytes and Hb SS erythrocytes was higher for suspensions in Hb SS plasma than for suspensions in normal plasma (Fig. 2): thus Hb SS plasma possesses the greater ability to induce erythrocyte aggregation. An increased ability to induce erythrocyte aggregation by Hb SS plasma is also to be expected from its elevated globulin concen-

TABLE IV

Rheology of Hb SS Erythrocytes: Deviation from Normal

	ISC	Non-ISC
Erythrocyte deformation		
Membrane flexibility	↓	↓
Internal viscosity	↑	—
Erythrocyte aggregation	↓	↓

tration (Table III). That the viscosity of suspensions of non-ISC in autologous plasma is normal at low shear rate (Fig. 6 A) despite the greater aggregating ability of Hb SS plasma indicates that non-ISC tend to aggregate less than do normal erythrocytes.

An estimation of aggregation tendency can be made by calculating the dependence (D) of viscosity on shear rates between 52 sec^{-1} and 0.052 sec^{-1} (45):

$$D = (\eta_B - \eta_A)/\eta_A$$

where η_A and η_B are the viscosity values at 52 and 0.052 sec^{-1} respectively. This shear dependence can be calculated for suspensions in plasma (D_P) and in Ringer's solution (D_R), and the difference ($D_P - D_R$) represents the contribution of erythrocyte aggregation to shear dependence of viscosity. As shown in Fig. 9, the values of $D_P - D_R$ for both non-ISC and ISC are lower than that for normal erythrocytes over the intermediate hematocrit range and hence the results show a reduced tendency for Hb SS erythrocytes to form aggregates.

General discussion. Findings reported here delineate a pathogenic role for oxygenated Hb SS erythrocytes in the microcirculation. ISC and, to a lesser extent, non-ISC appear to possess a lower than normal deformability in addition to a lessened tendency to aggregate (Table IV). Although a lessened tendency toward aggregation may promote blood flow in such vessels as postcapillary venules, erythrocyte deformation is the major flow determinant in capillaries where cells must pass through individually (43, 46). Therefore the altered membranes of Hb SS erythrocytes (ISC and non-ISC) and the elevated internal viscosity in ISC may impede passage through capillaries even under conditions of full oxygenation. The proposed importance of changed deformability of oxygenated Hb SS erythrocytes in affecting capillary flow is in agreement with the suggestion by Jandl, Simmons, and Castle (47) that the pathologic processes of Hb SS disease may be initiated in vessels admitting single cells only.

Fig. 3 shows that elevation of viscosity of oxygenated Hb SS blood over that of normal blood is mainly due to the presence of ISC. The difference in viscosity between oxygenated ISC in plasma and oxygenated non-ICS in plasma is 2-fold at 45% hematocrit and 5-fold at 90% hematocrit (Fig. 6 A). This difference approximates the increase in viscosity which occurs when Hb SS whole blood is partially deoxygenated by equilibration at a P_{O_2} of 40 mm Hg (7). At this P_{O_2}, the oxygen tension of normal mixed venous blood, approximately 60% of Hb SS erythrocytes are in the reversibly sickled form (7). It is likely that the ability to obtain pure preparations of ISC would have revealed an even greater difference in viscosity between the two cell types. It should not be inferred that the characteristics of ISC and non-ISC represent a discontinuous function. Presumably the membrane changes which lead to the formation of ISC are also present, but apparently to a lesser extent, in non-ISC. Clinical verification of the high risk properties of ISC exists in the recently found direct correlation between proportions of circulating ISC and shortening of red cell life span in Hb SS disease (48).

The present experiments with oxygenated Hb SS erythrocytes provide further refinements to the well-known viscosity cycle initiated by deoxygenation and therefore reversible sickling. As shear rate is lowered, Hb SS erythrocyte membranes undergo an abnormally pronounced reduction in flexibility; and the cells themselves, unlike reversibly sickled erythrocytes, tend to aggregate. These two alterations can be expected to generate a positive feedback cycle leading to flow stagnation. ISC contribute additional rheological hazards as a result of their high internal viscosity. Therefore all three rheological factors (internal viscosity, membrane flexibility, and erythrocyte aggregation) participate in the causation of erythrostasis, even under conditions of full oxygenation.

The significance of these experiments lies in their demonstration of and explanation of rheological changes in oxygenated Hb SS blood. Furthermore they provide fundamental information on basic mechanisms regulating blood viscosity, with emphasis on the relative importance of membrane flexibility versus the internal viscosity of erythrocytes. This approach to the study of rheological abnormalities in one hematological disorder might constitute a design for future rheological investigations into other disease states.

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