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Research Article

STUDIES ON THE DESTRUCTION OF RED BLOOD CELLS. XII. FACTORS INFLUENCING THE ROLE OF S HEMOGLOBIN IN THE PATHOLOGIC PHYSIOLOGY OF SICKLE CELL ANEMIA AND RELATED DISORDERS [1]

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The deoxygenation of blood from patients with sickle cell anemia leads to transformation of the red blood cells from biconcave discs into the bizarre elongated forms from which the disease derives its name (1). This change in morphology of the red cells is associated with increased viscosity of the blood proportional to the number of red cells so altered (2, 3). Much of the abnormal physiology of sickle cell anemia is attributable to this increase in the viscosity of deoxygenated blood (2–4) as well as to the greater mechanical fragility of the red cells that accompanies the sickled state (5–7). Thus, factors which impede the flow of blood may result in local hypoxia sufficient to increase the extent of sickling. The concomitant increase in viscosity of the blood further decreases blood flow so that local hypoxia becomes more marked, thereby increasing the degree of sickling. In this way the "vicious cycle" of sickle cell anemia (2, 3) is instituted, resulting in localized ischemia and ultimately thrombosis, with necrosis of tissue. The two chief features of sickle cell anemia are thereby explained, the chronic hemolytic anemia on the basis of the increased mechanical fragility and diminished life span of the erythrocytes *in vivo,* and the appearance of painful crises on the basis that various circumstances may temporarily so alter the flow of blood in an organ that the "vicious cycle" of erythrostasis, increased sickling, increased viscosity, etc., occurs and leads to localized ischemia or infarction (2).

Studies of the properties of hemoglobin solutions indicate that the sickling phenomenon is a resultant of the presence of S hemoglobin in the red cells of patients with sickle cell anemia (8, 9) and that the sickled cell may be considered to be a hemoglobin "tactoid" covered by a passively distorted cell membrane. Harris (8) has demonstrated that concentrations of S hemoglobin of at least 10 to 12 grams per cent are required for tactoid formation to occur upon deoxygenation of hemoglobin solutions and that this tactoid formation is associated with increased viscosity of the hemoglobin solutions. Indeed, at concentrations of S hemoglobin of 20 grams per cent or more, deoxygenation leads to gel formation.

These facts and in particular the contrast with the asymptomatic sickle cell trait suggest the desirability of defining in greater detail some of the quantitative aspects of the relationship between S hemoglobin and viscosity changes in whole blood and in solutions of hemoglobin in order that these may be related to physiologic events occurring during the course of the disease. The present study was undertaken in order to add to knowledge of such relationships.

CLINICAL MATERIAL

Twenty-one Negro patients with various hereditary hemoglobinopathies involving S hemoglobin were studied. The pertinent clinical and laboratory data are given in Table I. There were 8 cases of sickle cell anemia (hemoglobin phenotype: S hemoglobin), 9 of sickle cell trait (hemoglobin phenotype: A and S hemoglobins) and 3 of sickle cell-hemoglobin C disease (hemoglobin phenotype: S and C hemoglobins). Finally, in 1 patient 73 per cent of the hemoglobin was S hemoglobin, 21 per cent was A hemoglobin and 6 per cent was F hemoglobin. These findings are not characteristic of sickle cell trait as it is usually defined (10). Morphologically the red cells were normochromic and microcytic even after correction of pre-existing iron deficiency. This hematologic pattern has been called sickle cell-thalassemia disease by Singer, Singer, and Goldberg (11), but inasmuch as the patient's mother was hematologically normal and

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TABLE I

Clinical and laboratory findings in hereditary hemoglobinopathies

Patient	Age	Sex	RBC $\times 10^6$/ cu. mm.	HGB Gm. %	HCT %	MCV cu. μ	MCHC Gm. %	RETIC %	Icterus index	Per cent hemoglobin S	A	C	F	MCSHC Gm. %	Irreversibly sickled forms %	V_s/V_w*	Painful crises	Red cell half-life Days†	Aseptic necrosis of bone
R. W.	25	M	1.96	6.8	19.1	97	35	12.5	20	99	0	0	1.3	35	15.0	3.1	+		0
W. H.	18	M	2.96	8.1	23.0	78	35	8.2	100	96	0	0	3.6	34	16.7	2.8	+		0
Ir. H.	19	F	2.32	6.3	18.0	78	35	11.4	50	91	0	0	9.4	33	7.3	3.1	+		0
In. H.	15	F	1.73	5.6	17.9	103	31	14.1	12	98	0	0	1.7	31	6.4	3.2	+		0
C. Ho.	19	F	3.57	8.2	26.6	75	31	5.5	5	97	0	0	3.0	30	0.4	2.8	+	11	+
R. C.	37	M	3.65	10.0	33.2	91	30	3.7	12.5	95	0	0	4.7	29	3.6	3.3	+		+
E. S.	8	F	2.81	8.1	25.9	92	31	4.8	12.5	89	0	0	11.1	28	7.2	3.0	0		0
B. R.	13	F	3.54	7.7	26.0	73	30	11.2	12.5	90	0	0	10.0	27	6.6	3.2	+		0
B. C.	10	F	2.92	6.2	20.1	69	31	5.2	5	73	21	0	6.4	23	occ.	2.9	+		0
E. B.	30	F	2.93	8.5	24.8	84	34	6.7	5	52	0	47	1.2	18	0.2	2.5	0		0
C. D.	37	F	4.38	10.9	31.8	73	34	0.5	5	50	0	45	5.6	17	0	2.4	0	17	0
L. W.	28	F	4.00	10.8	36.8	92	29		10	52	0	47	0.7	16	1.0	2.4			
W. P.	94	M	3.36	10.6	33.6	100	32	1.7	5	48	51	0	1.5	15	0	1.8	0	22	0
P. H.	6	M	5.28	12.9	40.5	77	32		5	44	56	0	0.5	15	0	1.4	0		0
M. J.	60	F	4.74	13.0	39.7	83	32	2.6	5	45	55	0	0.8	14	0	1.2	0		0
C. Ha.	30	F		12.9	40.1		32	1.2	5	44	54	0	2.3	14	0	1.4	0	30	0
A. B.‡	62	F	2.70	6.7	21.2	79	32	1.5	5	44	55	0	0.9	14	0	1.5	0		0
G. P.	1	M	4.78	10.3	35.1	73	29	2.3	5	45	54	0	1.0	13	0	1.6	0		0
M. G.	23	F	4.79	9.4	32.2	67	29	1.4	5	39	60	0	1.2	11	0	1.2	0		0
B. T.	1	M	3.28	4.5	18.7	57	24	2.4	3	44	55	0	1.2	11	0	1.0	0		0
A. D.	1	F	6.02	10.2	36.2	60	28	0.9	4	35	60	0	4.9	10	0	1.0	0		0
Normal values:		M	5.0±0.3	14.8±0.8	45.9±2.7	91±7	32±2	1.0±0.5	6±2	0	99	0	0.9±0.4	0	0	1.0	0	30–33	0
		F	4.4±0.3	13.0±0.8	42.1±2.0	95±6	31±5												

* Viscosity at pO_2 = O/Viscosity at pO_2 = 680 mm. Hg.
† Cr^{51} method.
‡ Patient with chronic pyelonephritis and azotemia.

the father was not available for study, the genetic background of the abnormality in this patient could not be confirmed. For convenience, this patient will be designated as having S-A-F disease.

Whole blood will be described according to the contained hemoglobin, so that the designation S-S blood refers to whole blood taken from patients with sickle cell anemia, A-S to blood from patients with sickle cell trait, and so forth.

METHODS

Red blood cell counts, hematocrits, hemoglobin concentrations, mean corpuscular indices, icterus indices, reticulocyte counts and other hematologic determinations were made by standard methods (12). All hematocrit determinations were made on aliquots of whole blood after oxygenation in order to avoid errors introduced by the poor packing of red cells in the sickled form. The presence of the sickling phenomenon was detected by the metabisulfite method (13). The percentage of "irreversibly sickled" red cells (14), or cells which retain their distorted form in the presence of oxygen, was determined by counting the number of such cells per thousand red cells in smears of capillary blood from the ear lobe. The smears were made on cover slips and stained with Wright's stain.

Stroma-free solutions of hemoglobin were prepared by a modification of the method described by Drabkin (15). The modification consisted of using only physiologic saline as the wash fluid for the red cells, and of using the hemoglobin solutions after removal of the stroma by toluene, without crystallization of the hemoglobin. Methemoglobin concentration was measured by the method of Evelyn and Malloy (16), in all hemoglobin solutions, after viscosity measurements had been completed. Because methemoglobins, unlike oxyhemoglobins, do not form tactoids when exposed to low oxygen tensions, viscosity measurements are included in this report only if the percentage of methemoglobin was less than 4 per cent of the total hemoglobin concentration.

Paper electrophoresis of stroma-free hemoglobin solutions was carried out in an apparatus modified slightly from that described by Smith and Conley (17). The paper strips were scanned with a photoelectric densitometer [3] and the area under the resultant curves was measured with a polar planimeter in order to calculate the relative amounts of the various hemoglobins present. The mean corpuscular S hemoglobin concentration (MCSHC) of red cells was calculated by multiplying the mean corpuscular hemoglobin concentration (MCHC) by the percentage of S hemoglobin as obtained from analysis of the paper electrophoretic strips. The methods used for determining MCSHC are reproducible within a range of 5 to 10 per cent. Fetal hemoglobin was measured by the method of Singer, Chernoff, and Singer (18). The percentage of S hemoglobin in the red cells of patients with sickle cell anemia was calculated by subtracting the percentage of fetal hemoglobin present from 100 per cent.

[3] Photovolt densitometer, Model 52 C.

The survival time of labeled red cells in the circulation and sites of their sequestration were determined using radioactive chromium by the methods described by Jandl, Greenberg, Yonemoto, and Castle (19).

The viscosity of whole blood or of hemoglobin solutions was determined according to the methods of Harris, Brewster, Ham, and Castle (3) in Ostwald viscosimeters having bores of about 1 millimeter and flow times for water of 25 to 50 seconds at 37° C. Aliquots of oxalated bloods were equilibrated with gas mixtures containing known percentages of oxygen and nitrogen in the presence of 10 per cent carbon dioxide. Unless otherwise stated the viscosity of samples of whole blood was determined under the following "standard conditions": hematocrit adjusted to 35 per cent when the sample was oxygenated, pH 7.2 to 7.4, and temperature 37° C. maintained in a water bath. The pH of the blood was determined potentiometrically immediately after the flow time had been measured. In some experiments, the pH of whole blood was altered by adding 0.33 M HCl or 0.09 M $NaHCO_3$ prior to equilibration with the gas mixture. It was thus possible to vary the pH of the equilibrated blood from 6.8 to 7.5 without affecting greatly either the hematocrit or the mean corpuscular hemoglobin concentration.

RESULTS

Relation of hemoglobin concentration to viscosity of hemoglobin solutions

The viscosities of solutions of S hemoglobin and of A hemoglobin were determined after complete oxygenation and complete reduction, respectively. The changes in the relative viscosities that occurred in hemoglobin solutions whose concen-

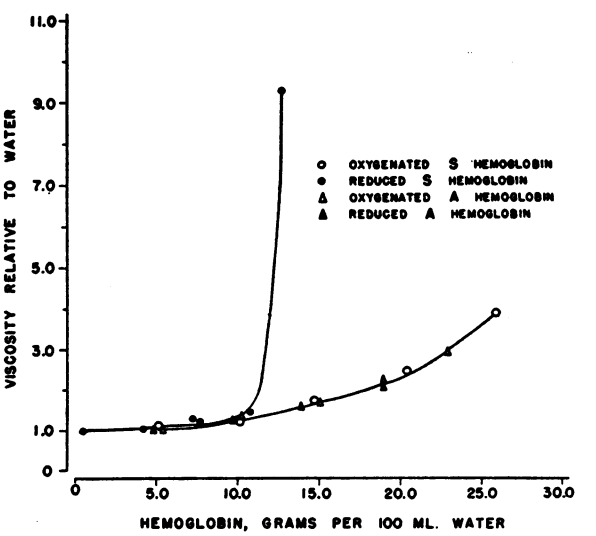

FIG. 1. THE EFFECT OF HEMOGLOBIN CONCENTRATION UPON THE VISCOSITY RELATIVE TO WATER OF STROMA-FREE HEMOGLOBIN SOLUTIONS

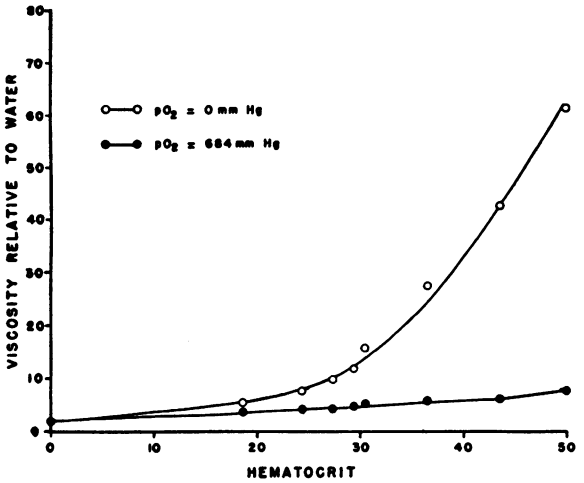

FIG. 2. THE EFFECT OF OXYGEN TENSION UPON THE INCREASE IN VISCOSITY THAT OCCURS WITH INCREASE IN THE HEMATOCRIT OF BLOOD FROM A PATIENT WITH SICKLE CELL ANEMIA

Note that the viscosity of reduced blood at an hematocrit of 25 per cent, an hematocrit frequently found to occur in patients with sickle cell anemia, is about the same as the viscosity of oxygenated blood at an hematocrit of 50 per cent. The curve shown here for oxygenated sickle cell anemia blood is not significantly different from the curve for normal blood. In the case of normal blood there is no difference between the curve for oxygenated blood and that for reduced blood.

trations varied from about 5 to 25 per cent are plotted in Figure 1. The values for reduced A hemoglobin, oxygenated A hemoglobin and oxygenated S hemoglobin fell along a common, gradually ascending curve. The values for reduced S hemoglobin also fell along the same curve for concentrations of less than 10 grams per cent but above this concentration the viscosity of reduced S hemoglobin rose precipitously to levels above those of even the most concentrated of the other hemoglobin solutions. Although not shown in Figure 1 because of the limitations of the scale, the viscosities relative to water of solutions of reduced S hemoglobin in concentrations of 15.2 per cent and 15.9 per cent were 61.2 and 72.2, respectively. At concentrations above 20 grams per cent, the solutions of reduced S hemoglobin were complete gels as observed by Harris (8).

Relation of hematocrit to viscosity of sickle cell anemia blood

Blood from a patient with sickle cell anemia was adjusted to various hematocrit values between 15 and 50 per cent by the addition or removal of autologous plasma. Aliquots of the blood at each hematocrit were fully oxygenated or completely re-

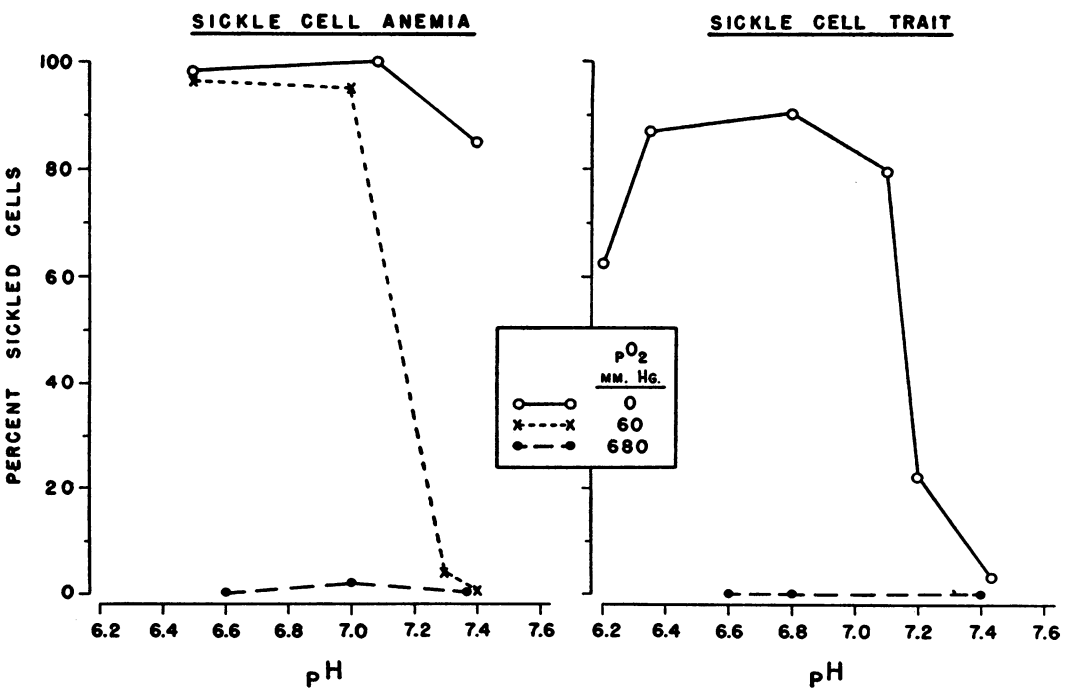

FIG. 3. THE EFFECT OF pH UPON THE SICKLING OF RED CELLS *In Vitro* AT DIFFERENT OXYGEN TENSIONS

duced by equilibration with appropriate gas mixtures. As shown in Figure 2, the viscosity of the oxygenated S-S blood increased slightly as the hematocrit increased. These values correspond with those for normal blood, whether oxygenated or reduced. There is no increase in the viscosity of normal blood upon deoxygenation. However, the viscosity of the reduced S-S blood increased much more rapidly than did that of the oxygenated aliquot especially at hematocrit values above 20 per cent. The figure shows that at a hematocrit of 25 per cent the viscosity of the completely deoxygenated S-S blood was approximately that of oxygenated blood at a hematocrit of 50 per cent.

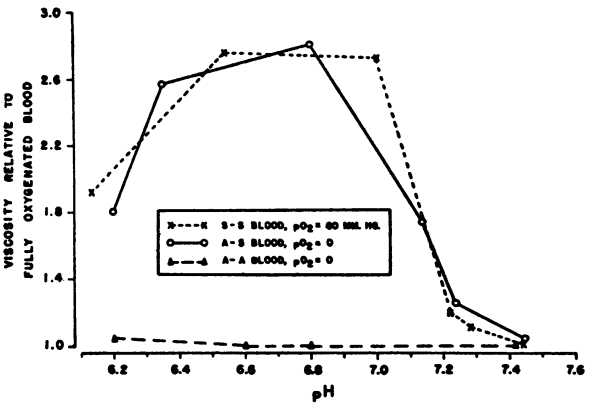

FIG. 4. THE EFFECT OF VARIATIONS IN pH UPON THE VISCOSITY OF WHOLE BLOOD

Effect of pH upon viscosity and the sickling phenomenon

The effect of change in pH upon the degree of sickling and the viscosity of S-S and A-S bloods at several oxygen tensions is shown in Figures 3 and 4. There were no significant effects of pH on viscosity or degree of sickling of S-S or A-S oxygenated blood. However, when the effects of pH were studied at oxygen tensions at which not all of the cells were ordinarily sickled, striking effects on the degree of sickling were observed (Figure 4). Thus, at an oxygen tension of 60 millimeters of mercury the viscosity and degree of sickling of S-S blood increased as the pH was lowered, until a maximum was reached at about pH 6.8 to 7.0. These changes in pH did not cause significant alterations in mean corpuscular hemoglobin concentration. For example, the MCHC was 31 per cent at pH 7.0 when the viscosity was high, and was 32 per cent at pH 7.3 when the viscosity had decreased.

As the pH of A-S blood was lowered to 7.0 at zero oxygen tension the viscosity of the blood approached the maximal values obtained for S-S blood and almost all the cells were sickled. This suggests that A hemoglobin may be incorporated into tactoids at low pH values and oxygen tensions. No effect of pH was observed on the viscosity of fully reduced A-A blood.

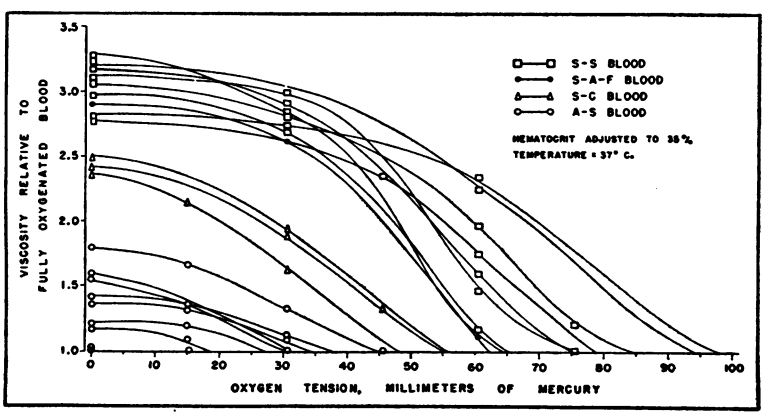

FIG. 5. THE EFFECT OF VARIATIONS IN OXYGEN TENSION UPON THE VISCOSITY OF WHOLE BLOOD OF PATIENTS WITH A VARIETY OF ABNORMAL HEMOGLOBINOPATHIES INVOLVING S HEMOGLOBIN

The hematocrit was adjusted to 35 per cent by the addition or removal of autologous serum in all cases, pH was maintained between 7.2 and 7.3 in all cases by maintaining a pCO_2 of 76 millimeters of mercury, and all observations of viscosity were made on blood at a temperature of 37° C.

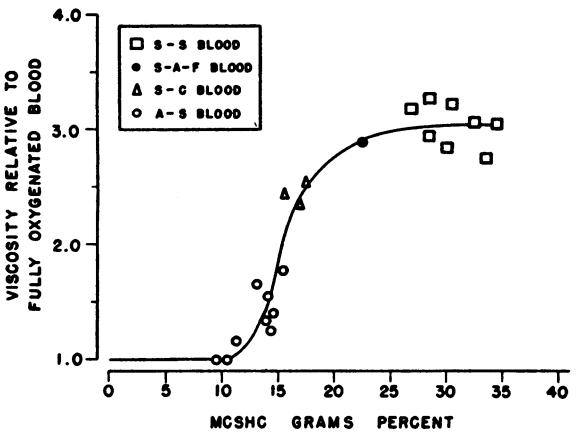

FIG. 6. THE RELATIONSHIP BETWEEN MEAN CORPUSCULAR S HEMOGLOBIN CONCENTRATION (MCSHC) AND THE VISCOSITY OF FULLY DEOXYGENATED BLOOD AT A HEMATOCRIT OF 35 PER CENT

Relation of S hemoglobin concentration within the red cells to the viscosity of whole blood

The alterations in viscosity of oxalated whole blood that occurred at various oxygen tensions under the standard conditions of hematocrit, pH and temperature referred to previously, are illustrated in Figure 5. The curves are based on observations made on the blood of each of the patients described in Table I. The viscosity at complete deoxygenation was greater for S-S blood than for A-S, or S-C bloods, but the viscosity of S-A-F blood fell within the range of S-S bloods. The oxygen tension that barely permitted sickling was also distinctly greater for S-S bloods than for A-S bloods, and was possibly greater than for the other variants of the S hemoglobinopathies. The viscosity curve of S-C blood fell between the curves that characterize S-S and A-S bloods. That of S-A-F blood was among those of the less viscous S-S bloods.

The data in Figure 5 suggest that the mean corpuscular S hemoglobin concentration (MCSHC) and the degree of change in viscosity upon deoxygenation are related phenomena. In Figure 6 is plotted the MCSHC of the red cells of each of the patients described in Table I in relation to the increase in viscosity that occurred when the corresponding blood was deoxygenated completely under the standard conditions of hematocrit, pH, and temperature. The resultant sigmoid curve is comparable to that in Figure 1, in which the viscosity of solutions of various concentrations of S hemoglobin is plotted. Both in the solutions and in the whole blood, and irrespective of the total MCHC, the first increase in viscosity occurred when the MCSHC was greater than 10 grams per cent. Solutions of reduced S hemoglobin became completely gelled at concentrations above 20 grams per cent. Similarly, in whole blood, there was no further increase in viscosity at MCSHC values above about 20 grams per cent, presumably be-

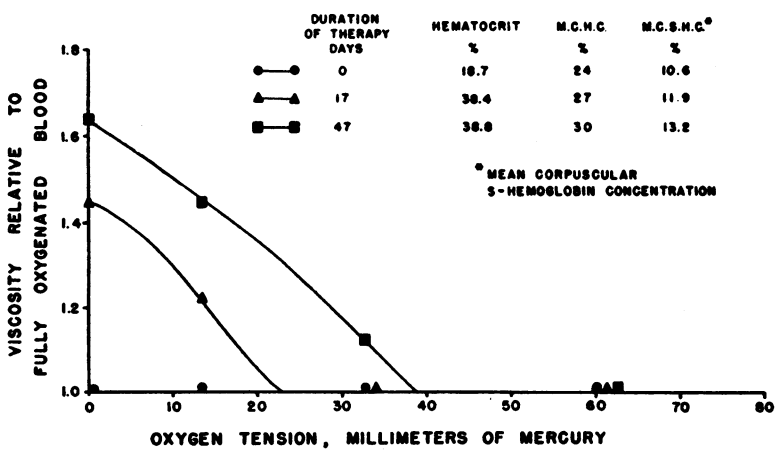

FIG. 7. CHANGES IN THE EFFECT OF DEOXYGENATION UPON THE VISCOSITY *In Vitro* OF BLOOD FROM A PATIENT WITH SICKLE CELL TRAIT (A-S BLOOD) AND COEXISTING IRON DEFICIENCY FOLLOWING TREATMENT OF THE PATIENT WITH FERROUS SULFATE

MCSHC is mean corpuscular S hemoglobin concentration.

cause all the hemoglobin within the red cells was completely gelled.

Further confirmation of the role of MCSHC in determining susceptibility to sickling of red cells came from observations on the bloods of two patients with sickle cell trait and coexisting iron deficiency. The data from one of these patients are presented in Figure 7. Initially, the MCHC was 24 grams per cent and the MCSHC was 10.6 grams per cent. At this time there was no increase in viscosity and no sickling of red cells upon complete deoxygenation of the blood under the standard conditions. However, paper electrophoresis and the metabisulfite tests indicated the presence of S hemoglobin. After iron had been given by mouth for 47 days the ratio of S to A hemoglobin within the red cells did not change, but, as the total MCHC rose, the degree of sickling and the corresponding changes in viscosity approached values commonly seen in sickle cell trait. An increase of MCSHC to 11.9 grams per cent when the MCHC was 27 grams per cent was sufficient to permit sickling after complete dexoygenation; this effect was still greater when the MCHC reached 30 grams per cent and the MCSHC was 13.2 grams per cent.

DISCUSSION

The importance of changes in the viscosity of whole blood *in vivo* in determining much of the pathologic physiology of the sickle cell syndromes (2, 3, 8, 20, 21) is supported by the present data. The S hemoglobin concentration is evidently the chief determinant in bringing about the changes that are observed *in vitro*. Thus, there are evident similarities between the curve of viscosity of whole blood plotted against the calculated mean corpuscular S hemoglobin concentration (Figure 6) and that of the viscosity of solutions of S hemoglobin plotted against concentration (Figure 1). Similarly, the point at which deoxygenation first alters viscosity is related to the concentration of S hemoglobin, whether in solution or in cells.

When the viscosity curves of the bloods of patients with various S hemoglobinopathies are compared, a similar relationship is seen between the mean corpuscular S hemoglobin concentrations and both the degree of viscosity change upon deoxygenation and the point at which deoxygenation first alters viscosity. The viscosity curves of the bloods from patients with various S hemoglobinopathies have recently been described by Harris, Brewster, Ham, and Castle (3) and by Griggs and Harris (20). Our observations are in agreement with theirs.

The dependence of the alterations of the viscosity curve upon the concentration of S hemoglobin suggests relatively little role for the other hemoglobins in the red cells of the patients under study. Evidence that other hemoglobins may react with reduced S hemoglobin has been presented by Singer and Singer (21). In the present observations the interaction of other hemoglobins with S hemoglobin was greatest at pH values lower than the usual physiologic range, as shown by the increased viscosity of fully reduced A-S blood as the pH was lowered below 7.4 (Figure 4). On the other hand, the viscosity changes that occurred in reduced A-S and S-C bloods at physiologic and higher pH values fell in the range that would be anticipated from the S hemoglobin concentrations alone. Thus, the interaction of A and C hemoglobins with S hemoglobin is probably minimal at normal physiologic pH values, but may play a role if intracellular pH is lowered. Conversely, the interaction of reduced hemoglobin molecules is diminished at pH values above 7.4 and even S-S interaction appears to be decreased slightly under such circumstances.

Alteration in pH is not the sole determinant of the extent of interaction among hemoglobin molecules, and it may be anticipated that other means for altering such interaction are present within the red cell. Thus, glutathione, which is present in increased amounts in the red cells of patients with sickle cell anemia (22) itself causes sickling and also reacts with the sulfhydryl groups of hemoglobin (23). Following treatment of the patient with adrenal steroids, decreases in the viscosity of S-S blood exposed to reduced oxygen tensions under the standard conditions were observed and were found to be associated with lowered blood glutathione levels (22). Similarly, there is evidence that there is an optimal concentration of potassium ions for sickling to occur under physiologic conditions (24).

The requirement of a mean corpuscular S hemoglobin concentration of approximately 10 grams per cent before sickling appears in deoxygenated

blood under standard conditions indicates the lower limit of sensitivity of the deoxygenation test for the sickling phenomenon. Thus, the blood of the patients with sickle cell trait and iron deficiency anemia did not sickle after deoxygenation until the MCSHC had increased to more than 10 grams per cent following the administration of iron (Figure 7). However, sickling of the bloods of the patients with iron deficiency was observed in the presence of metabisulfite even when the MCSHC was insufficient to permit sickling upon deoxygenation. The greater sensitivity of the metabisulfite test may be ascribed to the lowered pH at which the test is performed and perhaps to the possibility that strong reducing agents may increase the degree of interaction of hemoglobin molecules beyond that observed with deoxygenation alone. We have observed that when the MCSHC is reduced by suspending A-S cells in hypotonic solutions, with resulting dilution of cell contents by the entry of water, the lower limit of S hemoglobin concentration in the red cells that permits sickling with metabisulfite is about 7 grams per cent.

The determination of the limits of sensitivity of the usual screening tests for the presence of the sickling phenomenon poses certain problems. Since bloods with MCSHC values of less than 7 grams per cent do not sickle in the metabisulfite test and since this concentration of S hemoglobin approximates one-fourth to one-fifth of the total hemoglobin in a red cell, it follows that when S hemoglobin accounts for less than 20 to 25 per cent of the hemoglobin present in the fully hemoglobinated cell, the cells will not sickle. Hence it is not surprising that surveys of the ratios of S to A hemoglobins in patients with sickle cell trait have not uncovered instances in which less than 20 per cent of the hemoglobin was S hemoglobin (10). Yet, there is no apparent reason why individuals may not carry lower concentrations of S hemoglobin in their bloods. Such an eventuality would remain undetected because of the limitations of the sickling tests and the paper electrophoretic method as usually conducted. For example, Singer and Fisher (25) have shown by careful electrophoretic study of the blood of one patient whose blood did not sickle, that only 5 per cent of the total hemoglobin was S hemoglobin (MCSHC, 1.7 grams per cent). It may be anticipated that more sensitive methods will increase the number of instances in which S hemoglobin in amounts lower than 20 per cent of the total will be identified in the red cells of patients whose blood fails to sickle with metabisulfite. Conceivably, the present methods of detecting sickling can be modified to increase the likelihood of hemoglobin interaction, and hence to increase the sensitivity of such tests.

There is little difference between the viscosity of completely deoxygenated S-S blood at a hematocrit of 25 per cent and that of fully oxygenated blood at a hematocrit of 50 per cent (Figure 2). Thus, the physiologic consequences of sickling *in vivo* are presumably small except at sites of erythroconcentration. The spleen is such a site (2, 26); it is frequently enlarged, and may be a locus of distress in children with sickle cell anemia (27). The small fibrotic spleen characteristic of adult patients with sickle cell anemia is undoubtedly the end result of repeated episodes of splenic congestion and infarction. Again, patients with sickle cell trait or sickle cell-hemoglobin C disease, who are generally asymptomatic, may develop splenic infarction as a consequence of the hypoxia that may occur during travel at high altitudes (28). Similar adverse effects are to be anticipated as a consequence of hypoxia or of increased carbon dioxide concentrations in the blood, such as may occur during anesthesia.

The auto-splenectomy that occurs in adults with sickle cell anemia may act to the patient's advantage. Thus, the red cells of patient Co. Ho., whose spleen was not palpable, had a half-life of 11 days in the patient herself by the Cr^{51} method (19). The patient's labeled red cells tended to be sequestered in the liver, a site of low pressure blood flow, with no apparent concentration in the area of the spleen. However, when her labeled red cells were transfused into a normal recipient with an intact spleen, the half-life of her cells was only 6 days. In this recipient radioactivity appeared predominantly over the area of the spleen as well as that of the liver (19).

The clinical manifestations of the presence of S hemoglobin are related to the likelihood of sickling under physiologic oxygen tensions, which, in turn, appears to be dependent upon a sufficient intracellular S hemoglobin concentration. Thus, anemia, painful crises, and destructive bone

changes, such as aseptic necrosis of the head of the femur, occurred in our patients only in those with sickle cell anemia in whom the MCSHC was 28 per cent or higher. The patient with S-A-F disease in whom the MCSHC was 23 per cent also had both anemia and painful crises. No lesions of the bones were present but the patient is only 10 years old. Such lesions have been reported in other patients with S-A-F disease (29). Smith and Conley (30) have noted aseptic necrosis of the head of the femur in patients with sickle cell-hemoglobin C disease, but data permitting quantitation of the MCSHC in their patients were not published.

Mild anemia, without painful crises or bone lesions, was noted in our 3 patients with sickle cell-hemoglobin C disease, in whom the MCSHC was 16 to 18 per cent. In addition, the half-life of the red cells of one of these patients was found to be 17 days by the Cr^{51} method, which is appreciably shorter than the normal range of 30 to 33 days for this method. Similarly, one patient with sickle cell trait and an MCSHC of 15 grams per cent evidenced a moderate decrease in the red cell survival time, the half-life being 22 days. Other patients with sickle cell trait were not anemic unless a complication was present, such as iron deficiency (patients A.D. and B. T.) or uremia (patient A.B.) as noted in Table I.

One patient (C. Ha.) with sickle cell trait and an MCSHC of 14.1 grams per cent was observed during a severe attack of lobar pneumonia to have developed mild anemia and increased susceptibility to sickling of her red cells at physiologic oxygen tensions capable of initiating sickling of S-S blood. However, after recovery, the susceptibility of her red cells to sickling at such oxygen tensions diminished and their survival time *in vivo* became normal. The subsequent administration of cortisone failed to induce an increase in the susceptibility to sickling of her red cells.

The increased viscosity that occurs when red cells have been deoxygenated under standard conditions of hematocrit, pH and temperature supplies a simple method for quantitating the susceptibility to sickling of red cells. Many uses may be suggested for this technique. The viscosity responses may provide a means for following the altered susceptibility to sickling of red cells during treatment, as has been done here in patients with iron deficiency, or as has been done in studies of the effects of adrenocortical hormones in patients with sickle cell anemia (22). The viscosity curves have also been useful in patients with sickle cell anemia who have received transfusions of normal red cells. Under these circumstances many normal red cells are in the circulation and paper electrophoretic patterns of the hemolyzed blood may show relatively large amounts of A hemoglobin. However, because the patient's own red cells contain a high concentration of S hemoglobin, the viscosity of the blood under the standard conditions begins to increase at oxygen tensions above 40 millimeters of mercury, a finding that has not been observed in sickle cell trait. On the other hand, because the normal transfused cells do not sickle, the maximum viscosity of the blood under the standard conditions is not as great as that of the blood before transfusion. Finally, the viscosity curves are of value in following patients who develop painful crises. During a crisis and for variable periods of time thereafter, the blood of patients with S hemoglobin syndromes may evidence increased capacity to sickle under standard conditions at oxygen tensions intermediate between complete oxygenation and complete reduction (31).

Since this increased susceptibility to sickling of red cells can be reproduced simply by lowering the pH of the blood, it follows that acidosis may increase the degree of sickling *in vivo* and that alkalosis may reduce the magnitude of such changes. Indeed, it has been possible to induce painful crises in a patient with sickle cell anemia by administering acidifying agents, and to terminate the crises so produced, as well as spontaneous crises, by the administration of alkali (32).

SUMMARY

Twenty-one patients with various hereditary hemoglobinopathies involving S hemoglobin were studied with respect to the concentration of the different hemoglobins in their red cells, the clinical manifestations of the sickling phenomenon, and the changes in viscosity of their bloods upon deoxygenation *in vitro* under standard conditions of hematocrit, carbon dioxide tension and temperature.

A general relationship was demonstrated between the mean corpuscular S hemoglobin con-

centration (MCSHC) and the clinical manifestations of the S hemoglobinopathies. Patients in whom the MCSHC was less than 15 grams per cent were not anemic and had no painful crises. Those in whom the MCSHC was 15 to 18 grams per cent had evidence only of a mild hemolytic anemia. Significant anemia, bony lesions, and painful crises were limited to patients with MCSHC values of more than 20 grams per cent.

The effect of progressive deoxygenation upon the viscosity of whole blood at standard hematocrit and pH was also a function of MCSHC. In the pH range 7.2 to 7.4, the changes in viscosity upon deoxygenation were similar to those of solutions of S hemoglobin and viscosities rose sharply as the concentrations of S hemoglobin increased above 10 grams per cent. Similarly, less complete deoxygenation was necessary to elicit the first changes in viscosity at higher than at lower S hemoglobin concentrations.

Viscosity changes in bloods from patients with S hemoglobinopathies were also related to pH. Decreasing the pH of such bloods when partially deoxygenated led to increases in viscosity maximal at about pH 6.8 to 7.0. Conversely, raising the pH decreased the viscosity of partially deoxygenated blood. However, decreasing the pH of fully reduced S-S blood did not significantly increase its viscosity, whereas with fully reduced S-A blood the effect was striking. The evidence suggests that at pH values of 7.2 to 7.4, the hemoglobin concentrations are the major determinants of the changes in viscosity upon deoxygenation, but that at lower pH values S hemoglobin interacts with other hemoglobins.

The observations are consistent with the previously expressed concepts that the increased viscosity of blood that occurs upon deoxygenation is a major factor in the pathophysiology of the S hemoglobinopathies. From the *in vitro* data it may be inferred that the changes in viscosity are greatest at sites of erythroconcentration, and are augmented by lowering the pH and diminished by raising the pH of the blood.

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