Physical Properties of Red Cells as Related to Effects in Vivo. I. Increased Rigidity of Erythrocytes as Measured by Viscosity of Cells Altered by Chemical Fixation, Sickling and Hypertonicity

By Thomas Hale Ham, Rebecca F. Dunn, Richard W. Sayre and John R. Murphy

A SERIES OF STUDIES is reported on changes in physical properties of red cells that indicate abnormalities in their rigidity or deformability. Increased rigidity is a term used to describe changes in red cells leading to increased viscosity of flow, a shift to more Newtonian type of flow and lack of deformability as measured by packing under centrifugal force or by diminished flow through microfilters. Alterations in these physical properties will be related to changes in morphology, hemoglobin, stroma and to possible effects on the microcirculation and increased blood destruction.

In this first report, the rigidity of unmanipulated cells is compared to the rigidity of cells following fixation with glutaraldehyde or formaldehyde, sickling, hypertonic shrinkage and combined sickling and shrinkage. In a second report thermal treatment of red cells or products of hemolysis is related to increased viscosity and rigidity. A third report is concerned with the mechanism of increased destruction in vivo of heated cells. A hypothesis is developed that increased rigidity of red cells will result in abnormal blood flow in the microcirculation and in special circulatory beds, such as those of the spleen and kidney.
MATERIALS AND METHODS

Blood

Normal human or dog blood was defibrinated in flasks containing a gaseous mixture of 5 per cent CO₂ in air, to maintain the pH between 7.35 and 7.5. The serum and buffy coat were removed. In some experiments, the serum was replaced. In others, the cells were washed twice at 4 C. in three volumes of phosphate buffered isotonic NaCl at pH 7.4.(3) In studies of red cells at pH 8, blood was defibrinated in an open flask and washed twice in 3 volumes of saline solution. In studies of red cells in hypertonic media, the serum was mixed with solutions of sodium chloride, urea, or a combination of these, to give a final concentration of 10 per cent by volume of the aqueous phase and a predetermined hypertonicity when combined with the serum-red cell mixture.

Chemical Fixation

Glutaraldehyde, 1 per cent, in buffered 0.6 per cent sodium chloride was adjusted to pH 7.4 by titrating with 0.15 M NaOH, and kept at 4 C. protected from light. A mixture was also made of one part of aqueous formaldehyde, 40 per cent, and 9 parts 0.85 per cent sodium chloride buffered to pH 7.4. (4) The final mixture had a pH of 7.2 and was kept at room temperature. One part of washed, packed red cells was suspended in four parts of each fixative and incubated at 4 C. for 30 min. There was no difference in fixation when the ratio of fixative to packed red cells was varied over a range of 1:1 to 1:19. Chemical fixation by these agents is probably achieved by cross-linking intracellular and membrane proteins. Cell volume following fixation was determined by measuring the dilution of ¹³¹I albumin in suspensions of fixed and control cells.

Preparation of Samples

Stroma-free hemoglobin solutions were prepared in two ways to give a range of concentrations from 2 to 40 Gm. per cent. In the water-hemolysis method, the washed red cells were suspended in 9 volumes of water and the pH was lowered to 5.5 by bubbling CO₂ through the mixture, followed by centrifugation at 1,600 × G for 20 min. at 4 C. In the Singer method, in most instances, washed red cells in 1.5 volumes of water and 0.4 volumes of toluene were mixed vigorously, centrifuged 10 min. at 3,000 × G at 25 C. and the toluene-stroma layer removed. The hemoglobin solutions were recentrifuged at 17,000 × G to remove fragments of stroma and concentrated by pervaporation, blowing an air current across cellophane tubing containing the solution.

Red cell stroma was prepared in stainless steel test tubes (115 mm. × 20 mm.) by freezing washed red cells for 5 minutes at −75 C. and thawing for 6 minutes at 37 C. three times. The preparations were washed by suspension in 9 volumes of buffered isotonic NaCl and centrifuged at 17,000 × G at 4 C. Sonic treatment of red cells or stroma was performed in a Raytheon 10 kilocycle oscillator for 5 to 20 minutes at 10 C. After treatment, all samples were examined by phase microscopy for the presence of intact formed elements.

Reduction of blood or stroma-free hemoglobin solutions in 10–15 ml. volumes was done in a 250 ml. tonometer by equilibration for 30 minutes in a 37 C. water bath, using gaseous mixtures of 90 per cent N₂ and 10 per cent CO₂, to provide a pH of 6.6 to 6.7, or 90 per cent N₂ and 10 per cent CO₂, to provide a pH of 7.2 to 7.3. Samples were oxygenated with gaseous mixtures containing 95 per cent O₂ and 5 per cent CO₂ in a comparable manner.

Hemoglobin concentration was determined by the cyanmethemoglobin method. Hematocrits were determined by the method of Wintrobe and by the microhematocrit technique with centrifugation for 8 minutes. Estimates of erythrocyte deformability or rigidity were obtained by determining the flow of cell suspensions in serum or buffer salt solutions through microfilters of approximately 8 and 9 μ manufactured by the Millipore Corporation, Bedford, Massachusetts.
PHYSICAL PROPERTIES OF RED CELLS I.

Viscosity Determinations

Viscosity measurements at high shear rates were made with the Ostwald viscometer. The rates of flow were observed in a constant temperature water bath at 37 C. using 5 ml. samples of the various suspensions, comparing the flow rate to that of the suspension medium. Rates of flow were expressed as a ratio of that of the test substance to that of the medium. This procedure gave maximum but indeterminate rates of shear in of 1,000 sec.¹⁻⁶,⁷

Viscosity determinations at low shear rates, at 1.15 to 230 sec⁻¹, were made at 37 C. on a Brookfield cone-plate viscosimeter, model LVT 1/2 (Brookfield Engineering Laboratories, Inc., Stoughton, Massachusetts).⁸

Resistance to Packing

Whole blood was centrifuged at room temperature in Wintrobe hematocrit tubes for 35 minutes at 1,500 × G, and for an additional period of 20 minutes at 3,000 × G, with observations at 5 or 10 minute intervals. The data obtained were compared to a base line of the packed red cell volume observed by the microhematocrit, 8 minutes of centrifugation.

Mechanical Fragility

The mechanical fragility of red blood cells was determined by the method of Emerson et al.⁴ The hematocrits of the samples were observed but not adjusted.

RESULTS

A. Effect of the Fixatives

Red cells fixed in glutaraldehyde or formaldehyde were more viscous than normal cells (Figs. 1 and 2) and the flow properties were more Newtonian in behavior, i.e., less dependent on shear rate. Suspensions of normal red cells...
characteristically showed decreasing viscosity at higher shear rates, that is, non-Newtonian behavior. The glutaraldehyde-fixed cells did not flow through microfilters whereas fresh cells at similar concentrations flowed rapidly through such filters.

Fixed cells were not hemolyzed by three processes that readily destroyed untreated cells, namely, freezing and thawing, sonic vibration for 10 or 20 minutes and repeated washing in water. The fixed red cells maintained a normal morphologic appearance for many weeks in sealed slide preparations, kept in the room and examined by phase microscopy. When centrifuged at 1,500 and 3,000 × G, fixed red cells failed to pack (Fig. 3). Extreme degrees of agitation were required to resuspend the fixed cells after centrifugation. There

![Fig. 2. Viscosity measured at low shear rates (Brookfield viscometer) of red cells fixed in glutaraldehyde compared to samples suspended in isotonic sodium chloride. Two levels of hematocrit are shown.](image)

![Fig. 3. Resistance to packing of red cells fixed in glutaraldehyde compared to those in saline when centrifuged in Wintrobe tubes at different intervals of time and two gravitational forces. The microhematocrit method at 13,000 x G for 8 min. was used for measuring the control level of hematocrit.](image)
PHYSICAL PROPERTIES OF RED CELLS I.

Table 1.—Effect on Hematocrit, Mechanical Fragility of Red Cells and Viscosity of Normal Human Blood in Combinations of Different Concentrations of Sodium Chloride and Urea (Ostwald Viscosimeter).

<table>
<thead>
<tr>
<th>Concentrations of NaCl mOsm./Kg. H2O (dilution 10 per cent)</th>
<th>Concentrations of Urea mOsm./Kg. H2O (dilution 10 per cent)</th>
<th>Hematocrit per cent (change in Done minutes at 37C.)</th>
<th>Mechanical fragility, per cent</th>
<th>Hematocrit* per cent (change in medium= 1)</th>
<th>Viscosity* medium= 1 (change in per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>300</td>
<td>300</td>
<td>40.5</td>
<td>4</td>
<td>39.5</td>
<td>2.9</td>
</tr>
<tr>
<td>300</td>
<td>300</td>
<td>40.3</td>
<td>2.5</td>
<td>38.3</td>
<td>2.9</td>
</tr>
<tr>
<td>300</td>
<td>600</td>
<td>40.2</td>
<td>4</td>
<td>39.2</td>
<td>2.9</td>
</tr>
<tr>
<td>300</td>
<td>900</td>
<td>40.5</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>300</td>
<td>1200</td>
<td>42.8</td>
<td>3</td>
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</tr>
<tr>
<td>600</td>
<td>300</td>
<td>26.6(−34)</td>
<td>10</td>
<td>26.9(−32)</td>
<td>4.0(−38)</td>
</tr>
<tr>
<td>600</td>
<td>600</td>
<td>26.8(−29)</td>
<td>15</td>
<td>26.6(−32)</td>
<td>4.0(−38)</td>
</tr>
<tr>
<td>900</td>
<td>600</td>
<td>27.3(−33)</td>
<td>16</td>
<td>26.6(−32)</td>
<td>4.1(−39)</td>
</tr>
<tr>
<td>1200</td>
<td>26.2(−34)</td>
<td>59</td>
<td>23.2(−41)</td>
<td>6.4(−120)</td>
<td></td>
</tr>
<tr>
<td>(Hemolysis)</td>
<td></td>
<td>94</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*These observations were done at a different time on blood from the same subject.

were no signs of agglutination. Using albumin 131I as a tracer added to plasma, it was found that no net movement of water had occurred between the red cells and the supernatant solution when glutaraldehyde was added. Thus cell volume was unchanged although the apparent increase in hematocrit was 22 per cent.

B. Sickled Red Cells

It has already been demonstrated by Harris8 that stroma-free hemoglobin solutions from homozygous sickle disease form a viscous gel with tactoid formation when oxygen is removed. Further, the intact red cells of patients with homozygous and heterozygous sickle cell disease, when in the sickled form, show increased viscosity, resistance to packing by centrifugal force, and increased mechanical fragility.9,10 In the study reported here, the viscosity of sickled cells was greater than the viscosity of cells fixed with glutaraldehyde or formaldehyde (Fig. 1).

C. Effect of Hypertonicity on the Intact Red Cell

Suspending cells in hypertonic media decreased the hematocrit and increased mean corpuscular hemoglobin concentration (MCHC) (Tables 1, 2, 3). The addition of urea, 300 to 900 mOsm./Kg. of water, did not affect the hematocrit, as shown in Tables 1 and 2. The mechanical fragility and hematocrit were not changed when normal red cells were suspended in serum mixtures of NaCl, 300 mOsm./Kg. water, alone or in the presence of urea at concentrations of 300 to 1,200 mOsm./Kg. of water. Preliminary incubation at 37 C. for 60 minutes did not increase the mechanical fragility of those mixtures tested. The mechanical fragility of normal cells in hypertonic NaCl, 600 mOsm., 900 mOsm., and 1,200 mOsm., increased progressively and was not affected by
Table 2.—Effect on Hematocrit and Mechanical Fragility of Red Cells from a Patient with Sickle-Cell Trait* as Observed in Different Concentrations of Sodium Chloride and Urea.

<table>
<thead>
<tr>
<th>Concentrations of NaCl mOsm./Kg. H₂O (dilution 10 per cent)</th>
<th>Hematocrit per cent (change in per cent)</th>
<th>Mechanical Fragility per cent Done immediately at 37°C.</th>
<th>After 60 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>300</td>
<td>43.1</td>
<td>4.3</td>
<td>5.0</td>
</tr>
<tr>
<td>300</td>
<td>42.6</td>
<td>4.9</td>
<td>4.7</td>
</tr>
<tr>
<td>300</td>
<td>43</td>
<td>4.9</td>
<td>4.7</td>
</tr>
<tr>
<td>300</td>
<td>45.2</td>
<td>4.8</td>
<td></td>
</tr>
<tr>
<td>600</td>
<td>29.4 (−31.8)</td>
<td>21</td>
<td>15</td>
</tr>
<tr>
<td>600</td>
<td>31 (−28.1)</td>
<td>24</td>
<td></td>
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<tr>
<td>600</td>
<td>29.5 (−31.5)</td>
<td>27</td>
<td>21</td>
</tr>
<tr>
<td>900</td>
<td>26.5 (−38.5)</td>
<td>81</td>
<td></td>
</tr>
<tr>
<td>1200</td>
<td>26.4 (−38.7)</td>
<td>74</td>
<td></td>
</tr>
</tbody>
</table>

*Similar results were obtained with blood from a patient with homozygous sickle cell disease.

the presence of urea. Phase microscopy showed no change in the morphology of red cells suspended in 300 mOsm. of NaCl/Kg. water or upon the addition of urea. Similar results were observed using oxygenated blood from patients with sickle-cell trait and homozygous sickle cell disease (Table 2).

In oxygenated blood samples at 600 mOsm. of NaCl, some red cells were in the form of normal biconcave discs, but most were crenated with about an equal distribution of irregular (lumpy) discs, crenated (berry) cells and serrated (burr) cells. These morphologic changes occurred indistinguishably in samples of blood from normal human or canine subjects, or patients with sickle-cell trait (Figs. 4 and 5). No sickling was observed, and the crenated forms regained their normal biconcave disc-like shapes when resuspended in isotonic media at 300 mOsm. of NaCl. In oxygenated blood from homozygous sickle cell disease, there always were permanent sickled cells but no new sickled cells appeared in hypertonic solutions. At 900 and 1,200 mOsm. of NaCl/Kg. water, spindle forms became prominent in all samples including normal human and dog blood. These cells seemed flat and shrunk, with points often resembling sickled cells.

There was no change in morphology of normal red cells in isotonic or hyper-

Table 3.—Effect on Viscosity of Defibrinated Blood of Increasing Concentrations of Sodium Chloride, Comparing Intact Cells with Hemolysis Products Produced by Sonic Disruption.

<table>
<thead>
<tr>
<th>Concentration of NaCl mOsm./Kg H₂O (dilution 10 per cent)</th>
<th>Hematocrit per cent (change in per cent)</th>
<th>Mean corpuscular hemoglobin concentration of red cells (Gm./100 ml.)</th>
<th>Viscosity (medium = 1), per cent change*</th>
</tr>
</thead>
<tbody>
<tr>
<td>300</td>
<td>47.5</td>
<td>33</td>
<td>3.1 (−34)</td>
</tr>
<tr>
<td>600</td>
<td>31.5 (−34)</td>
<td>44</td>
<td>5.5 (+80)</td>
</tr>
<tr>
<td>1,200</td>
<td>25.8 (−46)</td>
<td>47</td>
<td>4.6 (+50)</td>
</tr>
</tbody>
</table>

*Hemoglobin concentration 15.6 Gm. per 100 ml. for each sample.
tonic media when oxygen was removed, but in isotonic media the red cells of sickle-cell trait and homozygous sickle cell disease changed to the classic sickled form. In 600 mOsm. of NaCl/kg. water, however, removal of oxygen from the crenated forms of sickle cells produced sickle processes that were short in length and few in number, but the cells were not deformed into the classic sickled form. The appearance of sickled processes was indirect evidence that tactoid formation occurred in the concentrated hemoglobin of the shrunken (crenated) sickled red cells.

Viscosity and resistance to packing of oxygenated cells. Oxygenated dog red cells, normal human cells, cells from patients with sickle-cell trait and homozygous sickle cell anemia showed the same normal viscosity (Ostwald viscosimeter) when suspended in serum to which 10 per cent by volume of isotonic NaCl was added (Fig. 6). When the NaCl was increased to 600 mOsm., the viscosity for the oxygenated cells rose (Figs. 6 and 7). Red cells in hypertonic NaCl showed a nearly constant viscosity as the shear rate was increased (Fig. 7). Conversely, red cells in isotonic media showed decreasing viscosity as the shear rate was increased. The viscosity curves actually crossed, indicating that the cells in hypertonic NaCl, even at a decreased hematocrit, had a greater viscosity than normal cells. Adding urea had no effect on viscosity. Increases in the tonicity to 900 and 1,200 mOsm. of NaCl further increased viscosity and the MCHC, as the red cell volume progressively shrank (Tables 1, 2, and 3). There was also an increased resistance to packing by centrifugal force in Wintrobe tubes (Fig. 8).

Viscosity of reduced blood in hypertonic media. Deoxygenation of normal human blood or dog blood had no effect on viscosity. When blood from patients
Fig. 5.—Phase microscopy of oxygenated sickle-cell trait cells at a concentration of 800 mOsm. of sodium chloride per kilogram of water showing irregular, crenated and serrated cells. No sickled forms occurred.

with sickle-cell trait or homozygous sickle cell disease was reduced at isotonic concentrations of NaCl, viscosity was elevated to a maximum degree, exceeding the effect of the 600 mOsm. media (serum with NaCl) upon normal cells. When the red cells from sickle-cell trait and homozygous sickle cell disease were first suspended in 600 mOsm. NaCl, and then reduced, however, the viscosity was maximal, as just described, or became unmeasurable in the Ostwald viscosimeter because the cells behaved like a gel. This was the only situation in which gel formation occurred. Reoxygenation of these samples eliminated the gel and decreased the viscosity to the value predicted from the level of tonicity. Accordingly, cells from patients with sickle cell hemoglobin behaved normally in hypertonic NaCl when oxygenated, but frequently gelled when reduced. The increased viscosity of cells in hypertonic NaCl was completely reversed when returned to isotonicity.

Crenation without hypertonicity. When normal human red cells were incubated at 37 C. for several hours in unbuffered isotonic saline at pH 8, 75 to 100
per cent changed to crenated forms comparable in appearance to those produced by 600 mOsm of NaCl (Fig. 4). There was no significant change in hematocrit, MCHC or viscosity associated with this increased crenation.

D. Viscosity of Hemoglobin, Stroma and Disrupted Red Cells

Stroma-free solutions of oxygenated hemoglobin from normal subjects and those with heterozygous sickle-cell trait behaved similarly at concentrations varying from 10 and 40 Gm. per cent. During the process of air drying, the concentration was readily increased to the range of 32 to 34 Gm. per cent. The preparation remained fluid and showed a gradual rise in viscosity (Fig. 9).

Fig. 6.—Effect on viscosity (Ostwald viscosimeter) of hypertonicity of oxygenated blood from normal human subjects, dogs and patients with sickle-cell trait or homozygous sickle cell disease. The viscosity is plotted against the observed hematocrit which was decreased in the hypertonic media.

Fig. 7.—Viscosity at low shear rates (Brookfield viscosimeter) for red cells in isotonic and hypertonic NaCl in serum. The number of red cells in each sample is the same.
Above 36 Gm. per cent, precipitation was detected on the surface of the cellophane tubing but these particles could be redissolved for a brief period. Viscosity measurements could be made in the Ostwald viscosimeter if these were carried out promptly and showed an abrupt rise (Fig. 9) as compared to lower concentrations. At a concentration of 40 Gm. per cent or higher, apparent gel
formation could not be prevented by continuing manipulation and the solutions gelled to a semisolid state on standing. On redilution with water the hemoglobin returned to a fluid state. There was no apparent effect on the viscosity of stroma-free hemoglobin solutions when the concentration of NaCl was varied from 300 to greater than 600 mOsm. Preparations of washed red cell stroma showed no change in viscosity when the NaCl concentration was adjusted to 300, 600, 900 and 1,200 mOsm.

Washed red cells were disrupted by sonic vibration in preparations containing NaCl concentrations of 300, 600 and 1,200 mOsm/Kg. water. The viscosity of the disrupted red cells was low and was not affected by differences in tonicity, in contrast to intact red cells (Table 3). In such experiments, the hemoglobin concentrations of the hemolysis products of the disrupted red cells were only 15.6 Gm. per 100 ml. of solution contrasted with hemoglobin concentration of the intact red cells which varied from 33 to 47 Gm./100 ml. of cells. When hemolysis products, made by sonic vibration, contained concentrations of hemoglobin of 30 Gm. per cent, they were too viscous to measure and they gelled at a concentration of 40 Gm. per cent.

**Discussion**

The flow characteristics of whole blood are described as non-Newtonian because the viscosity increases at lower shear rates. This is in contrast to the viscosity of a Newtonian fluid that remains constant, i.e., is independent of shear rate.

In the microcirculation the diameters of the vessels may be smaller or larger than the red cell. Bloch' and Guest et al. have demonstrated that during flow normal red cells may undergo changes in shape, be compressed, elongated, or twisted within milliseconds. Studying normal red cells at a constant hematocrit Fahraeus and Linquist and others demonstrated that the viscosity decreases when the shear rate rises, as occurs in capillary tubes of decreasing diameter. Although Mayer did not confirm the Fahraeus and Lindquist experiments he did not measure shear rates related to viscosity. The diminution in viscosity appears to be correlated with increased fluidity of the red cell when the shear rate rises. This property of increased fluidity is also characteristic of such gels as tobacco mosaic virus. The normal plastic red cells may decrease in viscosity when the shear rate rises and undergo remarkable shape changes during flow through the microcirculation.

According to the formulation by Dintenfass rigid red cells should behave in a more Newtonian manner, and be more viscous than normal cells because the rigid cells would have lost their normal plasticity or their capacity to become increasingly fluid. Increased rigidity was tested by observing red cells chemically treated with glutaraldehyde or formaldehyde. In this model it was evident that the red cells were fixed because they could not be hemolyzed by water, by freezing and thawing or by sonic vibration. There was evidence that the hemoglobin gelled and the stroma was rigid after this chemical fixation. The viscosity of the fixed cells was increased and more Newtonian. The glutaraldehyde-treated cells would not pass through a microfilter. The fixed red
cells did not change in volume but showed extreme resistance to packing by centrifugal force. Normal red cells, however, packed so well in the hematocrit tube that they were translucent to light whereas the fixed cells were not. This indicated that normal cells were so well adapted to each other by their changing shape that light was transmitted through the mass. Similar observations have been reported by Chien et al.\textsuperscript{17} for red cells hardened by acetaldehyde compared to normal cells.

The studies of sickled cells from patients with sickle-cell trait or homozygous sickle cell disease confirmed previous observations\textsuperscript{8,9} and serve as controls for observations in hypertonic media. Sickled cells were not fixed since they were hemolyzed by water, by freezing and thawing and by sonic vibration. Sickled cells showed the following characteristics: resistance to packing by centrifugal force, failure to pass through microfilters that readily permitted the flow of unsickled forms as described by Jandl et al.\textsuperscript{18} and an extreme increase in viscosity that was greater than observed for cells fixed in glutaraldehyde. It was concluded that sickled cells had lost fluidity and were therefore rigid, due to tactoid formation of the hemoglobin.\textsuperscript{8}

Stroma-free hemoglobin solutions of 40 Gm./100 ml., or above, showed gel formation. Hypertonicity did not change the viscosity of stroma-free hemoglobin solutions or the viscosity of washed red cell stroma. Increasing tonicity by the addition of urea did not alter the physical properties of cells nor were there shifts in water altering the MCHC. There was evidence, therefore, that all samples of red cells studied showed increased rigidity with loss of plasticity when exposed to hypertonicity from 600 to 1,200 mOsm. of NaCl/Kg. of water. This loss of deformability or increased rigidity appeared to result directly from the high concentration of hemoglobin within the cell. Under these conditions, loss of fluidity was probably due to a change in the physical properties of the hemoglobin, such as gel formation. This mechanism for increased rigidity has been suggested by Erslev and Atwater.\textsuperscript{19} Rand and Lacombe\textsuperscript{20} have observed that the viscosity of blood rose with increasing concentrations of mannitol, dextrose and hypertonic NaCl. They observed that hypertonic media produced crenation and increased hemoglobin concentration and related these changes to the increased viscosity. In extending their work, it was observed that crenation per se occurred in red cells incubated in isotonic media at pH 8 without a change in viscosity or MCHC. When increased viscosity occurred with crenation, it occurred with an increased concentration of hemoglobin.

In hypertonic media, the increase in viscosity, decreased filtration, resistance to packing and morphologic changes were identical for oxygenated blood from dogs, normal humans, patients with sickle trait and homozygous sickle cell disease. These results are at variance with the observations of Perillie and Epstein.\textsuperscript{21} These authors reported an increase in sickled forms in hypertonic solutions of sodium chloride, but no increase in the viscosity of normal cells or those from sickle-cell trait. They found an increase in viscosity only in cells from homozygous sickle cell disease in hypertonic media. The explanation for the differences in results is not clear. These authors have proposed that
hypertonicity in vitro resulted in sickling and that hypertonicity in vivo in the renal medulla and vasa recta would result in sickling in these areas of the microcirculation and thus lead to the renal disease in homozygous sickle cell disease. Whitten has studied the failure of renal concentrating capacity in homozygous sickle cell disease in response to the protein content of the diet. He believes the defect is a failure of the vasa recta and does not confirm the hypotheses of Perille and Epstein. It is possible, however, that new information submitted here may explain the abnormal function of the vasa recta as outlined in the following steps, to form a hypothesis.

It has been demonstrated that hypertonicity of sodium chloride resulted in increased viscosity of all cells but did not cause sickling of oxygenated blood. This increased viscosity could result in decreased flow in the vasa recta for all bloods, coincident with rising concentration of sodium chloride. The decreased rate of flow could lead to lowered pO₂ and pH and these in turn may result in sickling in both homozygous and heterozygous sickle cell disease. Sickling increases viscosity significantly and could affect the vascular bed of the medulla, accounting for thrombosis, papillary necrosis, hematuria and limited kidney function with hyposthenuria. The degree of sickling at lowered oxygen tension is variable and sickling takes a finite period. The rate of circulation through the kidney may affect the degree of sickling and the degree of obstruction in the microcirculation, and therefore produce a variable degree of renal involvement. Levitt et al. could reverse the renal defect of hyposthenuria in homozygous sickle cell disease by multiple transfusions of normal blood in children up to approximately 5 years of age but not in older children. Physiologically, this reversibility is consistent with normal functioning of the vasa recta when normal cells are made available to the kidney.

The red cells in sickle-cell trait are less susceptible to the sickling process than those of the homozygous disease. In sickle-cell trait, however, the increase in viscosity which results from hypertonicity at a normal hematocrit may slow the blood flow to such a degree that deoxygenation and lowered pH reach the critical level for sickling. Accordingly, the kidney presents a special situation in which the sickling process is enhanced, and as a consequence, patients with heterozygous sickle cell disease are vulnerable to renal complications, as are those with the homozygous disease.

**Summary**

Changes in the physical properties of red cells were produced by chemical fixation, the sickling process, by suspension in hypertonic sodium chloride and by a combination of hypertonicity and sickling.

These resulted in a loss of deformability or increased rigidity of cells as indicated by a rise in viscosity of cell suspensions and hemolysates, more Newtonian flow, diminished filtration of cell suspensions through microfilters, resistance to packing by centrifugal force and abnormal susceptibility to mechanical trauma. The results of these studies show that in hypertonic sodium chloride all cells have an increased viscosity and are more rigid. The relationship of these physical properties to the behavior of cells in the micro-
circulation is discussed for normal subjects and those with sickle cell disease, including the effect on the kidney.

SUMMARIO IN INTERLINGUA
Alterationes in le proprietates physic del erythrocytos esseva producite per fixation chemic, per le processo falcificatori, per suspension in hypertonic chloruro de natrium, e per un combination de hypertonicitate e falciformation.

Le proceduras resultava in un perdita de deformabilitate o un augmentate rigiditate del cellulas, manifeste in un augmentate viscositate de suspensions e hemolysatos cellular, un intensificate fluxo newtonian, un reducite filtration de suspensions cellular a transverso microfiltros, resistentia contra paccage per fortias centrifuge, e un susceptibilitate anormal pro trauma mechanic. Le resultatos de iste studios monstra que in hypertonic chloruro de natrium omne le cellulas ha un augmentate viscositate e es plus rigide. Le relation inter iste proprietates physic e le comportamento del cellulas in le microcirculation es discutite pro subjectos normal e pro subjectos con morbo a cellulas falciforme, incluse le effecto super le renes.

ACKNOWLEDGMENTS
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REFERENCES
Physical Properties of Red Cells as Related to Effects in Vivo. I. Increased Rigidity of Erythrocytes as Measured by Viscosity of Cells Altered by Chemical Fixation, Sickling and Hypertonicity

THOMAS HALE HAM, REBECCA F. DUNN, RICHARD W. SAYRE and JOHN R. MURPHY