The Effect of 2,3-DPG on the Sickling Phenomenon

By E. Beutler, N. V. Paniker and C. West

Red cells were depleted of 2,3-diphosphoglycerate (2,3-DPG) by storage in acid-citrate-dextrose (ACD) solution. No change in the sickling-oxygen saturation curve was observed. 2,3-DPG did not affect the viscosity of deoxygenated sickle hemoglobin solutions. It appears, then, that the interaction between 2,3-DPG and sickle hemoglobin does not produce configuration changes which influence the aggregation leading to sickling.

The unique chemical characteristics of sickle hemoglobin results, upon deoxygenation, in molecular aggregation and consequent deformation of the red cell. Although there is some question about the exact mechanisms involved in the formation of intermolecular bonds, there would seem to be little doubt that a high degree of configurational specificity is required for sickling to occur. Recent studies of the interaction between 2,3-diphosphoglycerate (2,3-DPG) and hemoglobin have suggested that the binding of 2,3-DPG to hemoglobin results in a configurational change decreasing the affinity of hemoglobin for oxygen. It appeared possible to us that this configurational change might, in the case of sickle hemoglobin, also effect the sickling process. Such an effect of 2,3-DPG would have obvious therapeutic implications. For this reason we have investigated the effect of 2,3-DPG levels on the tendency for sickle cells to sickle and for 2,3-DPG to influence the viscosity change of 2,3-DPG upon deoxygenation.

Materials and Methods

Blood for these studies was obtained from several untransfused patients with sickle cell disease. To determine the extent of sickling of a blood sample a 33.3 per cent suspension of packed red cells was prepared in a thawed aliquot of fresh-frozen type-compatible serum. The suspension was equilibrated for 10 minutes in a tonometer with a gas mixture containing 5 per cent CO₂ and variable proportions of oxygen and nitrogen. A solution containing 5 per cent formalin and 0.01 M Na₂HPO₄ in 0.9 per cent saline was equilibrated with the same gas mixture. A sample of the red cell suspension was drawn into a plastic syringe containing equilibrated formalin solution. After 5 minutes of fixation, the cell suspension was introduced into a hemocytometer counting chamber and the percentage of sickled cells enumerated using a criteria described previously. Oxygen dissociation curves were determined using five different mix ratios according to the mixing technique described by Edwards.
EFFECT OF 2,3-DPG ON SICKLING

Fig. 1.—The relationship between per cent saturation of hemoglobin with oxygen and the percentage of cells sickled in fresh blood and stored (2,3-DPG depleted) blood from an SS homozygote. The methods used are described in the text.

In order to estimate the effect of 2,3-DPG on viscosity of the hemoglobin solutions a hemolysate was prepared from blood which had been stored in ACD solution for 2 weeks. Packed washed red cells were lysed by freezing and thawing, the hemoglobin concentration was adjusted to 25 cm. per cent, and the hemolysate centrifuged at 5000 × g. for 10 minutes. The concentration of hemoglobin was adjusted to 15 cm. per cent and sufficient phosphate buffer was added to give a final phosphate concentration of 0.01 M and a pH of 7.4. The hemoglobin solutions were placed in tonometers, and deoxygenated with nitrogen or fully oxygenated with air for 60 minutes at 37°C. Varying degrees of oxygenation were achieved by mixing appropriate volumes of deoxygenated and oxygenated hemoglobin solutions. The solutions were introduced into a Gilmore No. 1 falling ball viscometer under ligroine (Eastman Organic Chemicals) and the time required for the ball to move 10 cm. at 25°C was recorded.

RESULTS

The relationship between the degree of sickling and the per cent saturation of hemoglobin with oxygen is presented in Fig. 1. In plotting these data the per cent saturation at the various percentages of oxygen used has been computed from the oxygen dissociation curve which, as was to be expected, progressively shifted to the left during the storage of blood in ACD solution. It is apparent from these data that no major alteration in the extent of sickling occurred as the red cells lost their 2,3-DPG during storage. However, blood storage produces many changes in the erythrocytes aside from loss of 2,3-DPG. Thus, it is possible that other alterations, such as change in intracellular sodium and potassium concentrations or the concentrations of adenine nucleotides might affect the sickling process. In order to determine whether 2,3-DPG itself had an effect, the effect of deoxygenation on viscosity of hemoglobin solutions was also investigated. Figure 2 shows the effect of deoxygenation upon the viscosity of solutions consisting predominantly of (1) hemoglobin A and of (2) hemoglobin S with and without added 2,3-DPG. As has been shown previously by others, deoxygenation produced a marked increase in the viscosity of solutions of sickle hemoglobin, but had no effect on the viscosity of solutions of hemoglobin A. The alteration of the viscosity of hemoglobin S solutions was not affected by the addition of 2,3-DPG.
An incidental, as yet unexplained, observation made in the course of these studies was that aging of hemoglobin solutions at 4°C resulted in loss of their capacity to undergo gelling when deoxygenated. This phenomenon was not due to the formation of methemoglobin, and it must be assumed that minor configurational changes during storage impaired the aggregation of deoxy sickle hemoglobin molecules. It is of interest, in this respect, that sterile incubation of blood at 37°C in the presence of oxygen caused loss of the ability of red cells to sickle when subsequently deoxygenated.7

These studies demonstrate that the changes in configuration of hemoglobin which occur as a result of 2,3-DPG binding do not seem to increase or decrease substantially the tendency for hemoglobin S to gel or for hemoglobin S-containing red cells to sickle when deoxygenated.

REFERENCES

The Effect of 2,3-DPG on the Sickling Phenomenon

E. BEUTLER, N. V. PANIKER and C. WEST

Updated information and services can be found at:
http://www.bloodjournal.org/content/37/2/184.full.html
Articles on similar topics can be found in the following Blood collections

Information about reproducing this article in parts or in its entirety may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at:
http://www.bloodjournal.org/site/subscriptions/index.xhtml