

## THE FUNCTIONAL PROPERTIES OF SICKLE CELL BLOOD

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### 1. Introduction

It is known that whole blood from patients with sickle cell anemia has a decreased oxygen affinity [1,2]. Purified hemoglobin S solutions, however, are normal [3,4]. A systematic study of the effect of 2,3-DPG and of other known allosteric regulators of oxygen affinity of HbS in whole blood seems desirable, indeed essential, to understand the reason for the altered oxygen affinity of the hemoglobin S molecule in the intact red cell environment. A more complete understanding of the functional properties of SS-blood would also be important to assess the physiological significance of such an alteration, and to evaluate the therapeutic effect of cyanate administration.

### 2. Materials and methods

The apparatus shown in fig.1 has been specially developed to obtain oxygen dissociation curves (ODC) of microsamples (approx. 390  $\mu$ l) of whole blood, or concentrated hemoglobin solutions, under strictly controlled experimental conditions of pH,  $P_{CO_2}$ , temperature, etc. The ODC apparatus shown allows a twenty-fold sample economy over previously described methods [5,6]. Its use can readily be extended to the study of other pathological hemoglobins, or blood samples in short supply. ODCs

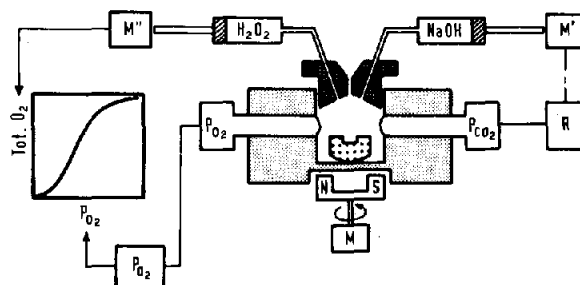


Fig.1. Apparatus for the determination of oxygen dissociation curves of whole blood, or concentrated Hb solutions. The blood sample (approx. 390  $\mu$ l) is first deoxygenated at the required  $P_{CO_2}$  in a tonometer at 37°C. The deoxy blood is then gradually oxygenated by  $H_2O_2$  addition at constant speed. The total amount of oxygen added to the cuvette is then plotted against the value of  $P_{O_2}$ . To prevent undue change of pH and  $P_{CO_2}$ , the  $P_{CO_2}$  value is continuously monitored and kept constant by the automatic addition of base. Methemoglobin formation is prevented by the addition to the whole blood of 1 to 2  $\mu$ l of a concentrated catalase preparation.

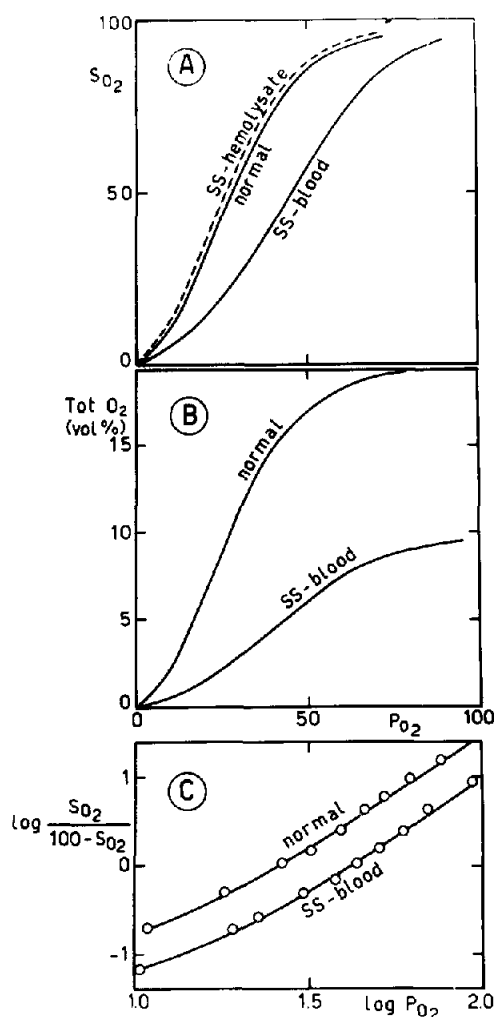
obtained by this new method have been carefully compared with oxygen binding data determined by classical manometric procedures, and found to be in very close agreement. A detailed description of this instrument will appear elsewhere [7].

Samples (1 to 2 ml) of whole sickle cell blood were obtained from a 29 year old, sickle cell homozygote, male subject; and used within 2 h. A total of 14 ml of whole blood, over a period of six days, was needed to

obtain all the data reported in the present paper. Thus, the limited amount of blood withdrawn was of no significance to the health condition of the severely anemic subject investigated. HbS concentration was determined on hemolysates by electrofocusing [8], and found to be in excess of 90% of total hemoglobin. Similar confirmatory studies on the functional properties of SS-blood, but more limited in regard to the range of experimental conditions, have been made on four more blood samples (1 to 2 days old) obtained from homozygote sickle cell subjects.

### 3. Results

Fig.2 shows the ODC of normal and sickle cell



blood at  $P_{CO_2} = 40$  mm Hg,  $pH \sim 7.4$ . The two curves have been represented as % saturation (fig.2 A) and as total oxygen content (Fig. 2 B) vs.  $P_{O_2}$ . This last form of representation is much more useful for physiological considerations. A separate series of experiments proved that the curves of Fig.2A and B truly represent equilibrium conditions. The experiments involved equilibration of completely oxygenated SS-blood with various partial pressures of oxygen in a microtonometer for 30 min, followed by measurement of total oxygen by a micromanometer technique. Fig.2C shows Hill's plots of the oxygen binding data. It is remarkable to find that up to the high oxygen saturation range investigated (approx. 96%), the value of  $n$  seems to be identical for SS and normal blood. The ODC of sickle cell hemolysate (broken line of Fig.2A), which was obtained by freezing and thawing a sample of SS-blood, is seen instead to be very close to the ODC of normal blood.

Fig.3A shows that the logarithm of the oxygen pressure required for 50% saturation ( $\log P_{50}$ ) changes with pH to about the same extent in SS= blood as in normal blood. Fig.3A also shows that the  $\Delta \log P_{50}$  between blood with normal and zero 2,3-DPG content is the same for normal and SS-blood. Fig.3B shows microtitration experiments at constant  $P_{CO_2}$  for oxy and deoxy SS-blood. The vertical distance between the two curves at constant pH is a direct measure [9] of the Bohr protons release upon deoxygenation (and sickling) of the hemoglobin S molecule in the intact erythrocyte. At  $pH = 7.4$ , the amount of Bohr protons released per oxygen molecule combined is approx.  $0.40 \text{ mEq } H^+$ , which is in satisfactory agreement with the value of  $(\Delta \log P_{50} / \Delta pH)$  obtained from the effect of pH on the oxygen affinity (fig.3A). Fig.3C shows the total  $CO_2$  content of oxy and deoxy SS-blood at constant  $P_{CO_2}$ . The vertical distance between the two curves, at  $pH 7.4$ , corresponds to a release of  $0.08 \text{ mEq } CO_2$  per mEq oxygen combined at constant pH. This

Fig.2. (A) Oxygen dissociation curves of normal, SS-blood, and SS-hemolysate at pH 7.4, 7.38, and 7.26 at  $37^\circ C$ . (B) ODC of normal and SS-blood expressed as total oxygen in solution vs.  $P_{O_2}$ . (C) Hill's plots of the ODC for normal and SS-blood. The Hill's coefficient  $n = 2.5$  for both blood samples.

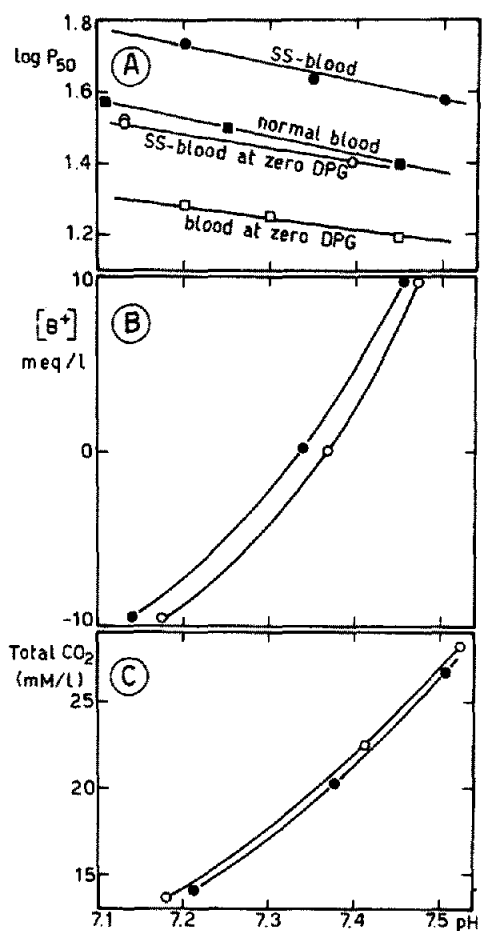


Fig. 3. (A)  $\log P_{50}$  values vs. pH for normal and SS-blood at normal and zero, 2,3-DPG levels. (The zero 2,3-DPG conditions have been obtained by incubating whole blood at 37°C for 8–12 h.) (B) Titration curves of deoxy (○) and oxy (●) SS-blood at constant  $P_{CO_2} = 40$  mm Hg,  $t = 37^\circ C$ . (C) Total  $CO_2$  vs. pH for deoxy (○) and oxy (●) SS-blood at constant  $P_{CO_2} = 40$  mm Hg,  $t = 37^\circ C$ .

value is practically the same as that found for normal blood [10].

#### 4. Discussion

The results presented in this report indicate that the large shift to the right of the ODC of SS-blood cannot be due to an abnormal effect of either pH,  $P_{CO_2}$ , or 2,3-DPG, or to an intrinsically altered

oxygen affinity of HbS. This is clearly shown by the normal oxygen affinity of SS-hemolysate (Hb concentration approx. 7%) in the presence of  $CO_2$ , 2,3-DPG, and all other red cell components (fig. 2A). The results of the microtitration experiment (fig. 3B) also rule out significant changes (i.e., greater than 0.05 to 0.1 pH units) in the intracellular pH of deoxy SS-erythrocytes upon sickling. In fact, if the changes in  $\log P_{50}$  found for SS-blood were due to a decrease in the intracellular pH, such a decrease should then be in the range of 0.6 pH units. At constant  $P_{CO_2}$ , however, the buffer power of the intracellular fluids is in the order of 190 mEq protons per pH unit. Thus a liberation of approx. 110 mEq protons per liter (against an experimental value of approx. 2.0 mEq/liter) upon sickling would be required to explain the change in oxygen affinity found for SS-blood.

It has been previously reported that both a decrease of pH or an increase of 2,3-DPG favors gelation, as shown by a decrease in the minimal gelling temperatures of concentrated HbS solutions [11]. The reverse effects, i.e., liberation of protons upon sickling and an increased effect of 2,3-DPG, are probably too small to be detected by the experimental approach in this paper. They are clearly of no significance in explaining the altered oxygen affinity of SS-blood. Oxygenation of a purified hemoglobin solution causes, at physiological pH values, a release of protons from imidazole 146 $\beta$  [12] and valine 1 $\alpha$  [13]. In presence of  $CO_2$  and organic phosphate, however, the net amount of protons released upon oxygenation changes as the result of: (a) hydrogen being released due to the Bohr effect, (b) hydrogen ion uptake due to the disappearance of oxy-labile carbamino compounds, and (c) hydrogen ion liberation due to the breaking of hydrogen bonds between negatively charged groups of 2,3-DPG and positively charged groups of hemoglobin [14]. The finding of a normal Bohr effect in whole SS-blood suggests that the ionisation of all the 'Bohr' groups (either in the oxy- or deoxyhemoglobin molecule) is not affected by sickling.

The most likely explanation for the large change in oxygen affinity in whole SS-blood can thus be attributed to the formation of aggregates of deoxygenated, or partially deoxygenated HbS molecules inside the red cell. This deduction is in complete agreement with the conclusions previously reached

[15,16] on SS-erythrocytes suspended in isotonic phosphate buffers, but no reasonable explanation has yet been given in molecular terms for this major effect.

It would be interesting to analyse the data shown in figs.2 and 3 according to the allosteric theory of hemoglobin function. Unfortunately, an analysis of this kind, analogous to that made on homogeneous hemoglobin solutions [17], is not feasible for SS-blood. In fact, it has been shown [18,19] that a certain proportion of circulating erythrocytes in a given SS-patient are irreversibly deformed. Such erythrocytes have a much higher hemoglobin content and show a greatly decreased oxygen affinity. SS-blood is thus not a homogeneous system in regard to oxygen affinity. An analysis of the top and the bottom of the ODC, as required for a proper calculation of the allosteric parameters of the MWC theory is thus meaningless. A definite answer as to the reason for the altered oxygen affinity found for SS-blood might be obtained instead from an accurate study of the oxygen binding data of solutions of SS-hemoglobin at various concentrations.

The large shift of the ODC of SS-blood deserves additional consideration from a more physiological standpoint. Fig.4 shows the decrease in oxygen saturation and the total amount of oxygen delivered by normal and SS-blood with decreasing  $P_{O_2}$ . All the lines shown have been calculated from the ODCs reproduced in fig.2A and B. The basal oxygen

requirement at rest, for the SS-subject investigated, was 220 ml/min. If the same subject had a normal cardiac output of 5000 ml/min, the total oxygen transported by the SS-blood *in vivo* would be  $5000 \times 0.095 = 495$  ml  $O_2$ /min. (The factor 0.095 is the oxygen capacity of the SS-blood of fig.2B in ml oxygen per ml blood.) Of this total amount 220 ml are required at rest, leaving an oxygen saturation in the mixed venous blood of *ca* 45% and a  $P_{O_2}$  of 40 mm Hg.

It is remarkable to note (fig.4) that at this  $P_{O_2}$  the SS-blood, by virtue of its greatly shifted ODC, can actually deliver more oxygen than the normal blood with twice its oxygen capacity. The large shift in the ODC of SS-patients can thus be considered a powerful compensatory mechanism in increasing the efficiency of the oxygen delivering system. This is again a beautiful example of a compensatory physiological phenomenon mediated by a molecular mechanism.

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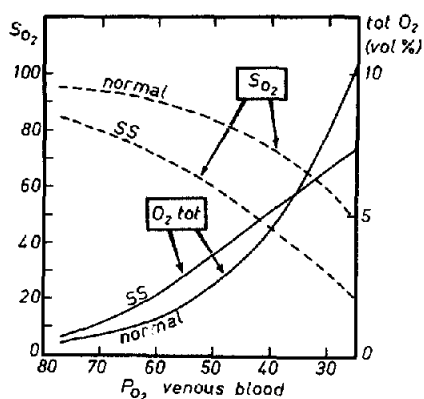


Fig.4. Decrease in oxygen saturation, and total oxygen released with decreasing  $P_{O_2}$  for normal and SS-blood at  $P_{CO_2} = 40$  mm Hg,  $pH = 7.4$ .

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