The Relative Effect of 2,3-Diphosphoglycerate on the Oxygen Affinity of Fetal and Adult Hemoglobin in Whole Blood

A number of organic phosphates present in the red blood cell (RBC) and particularly 2,3-diphosphoglycerate (DPG) are capable of binding to reduced hemoglobin (Hb), thus decreasing its affinity for oxygen (O_2)[1,2]. In vitro experiments with hemoglobin solutions have shown that the affinity of DPG for fetal hemoglobin (HbF) is considerably less than for adult hemoglobin (HbA)[3]. As a result, the effect of DPG on the O_2 affinity of HbF is significantly smaller than on HbA. Using solutions of pure HbF and HbA, TYUMA and SHIMIZU[4] and BUNN and BRIEHL[5] demonstrated that the effect of DPG on the O_2 affinity of HbF is approximately 40% of that of HbA. The smaller effect of DPG on the O_2 affinity of HbF (as compared to HbA) has also been confirmed in whole blood[6,7] and provides a logical explanation for the higher O_2 affinity of fetal blood when compared with adult blood.

On the basis of these findings, one would expect that the O_2 affinity of blood containing significant levels of HbF should be related to the relative proportions of HbF and HbA and to the level of DPG.

Previous attempts to correlate the O_2 affinity of cord blood with the level of HbF have resulted in conflicting results[8,9]. However, these experiments were performed at a time when the role played by DPG was not known. More recent evidence indicates that the oxygen affinity of blood from human infants is related to the concentration of HbA and to the level of DPG[10].

The present investigation was undertaken in order to define the relative effect of DPG on the O_2 affinity of HbF and HbA in the intact RBC – both in vitro and in vivo.

Two groups of experiments were performed. In the first group of experiments venous blood from 4 normal, non-smoking adults and cord blood from 6 normal infants of different gestational ages were collected in heparin. O_2 affinity, hematocrit (Hct%), hemoglobin (Hb g/100 ml), fetal hemoglobin (HbF%) and red cell DPG (nmoles/ml RBC) were measured on each sample of blood immediately after collection. Part of the blood was then left at ambient temperature for up to 24 h in order to produce a fall in DPG, and the measurements were repeated at various intervals of time. A total of 27 determinations was performed on the 10 samples of blood.

In the second set of experiments, O_2 affinity, Hct, Hb, HbF and DPG were measured on venous blood from 25 normal, non-smoking adults and on cord blood from 40 normal infants. Infants with widely different gestational ages (28 to 43 weeks) were purposely included in the study in order to obtain bloods with different concentrations of HbF (59–99%).

Blood O_2 affinity was determined in duplicate by the 'mixing technique' described by EDWARDS and MARTIN[11] and was expressed as P 50, i.e. the PO_2 at O_2 saturation = 50%, pH = 7.40 and temperature = 37 °C. The pH, PO_2 and PCO_2 of blood 50% saturated with O_2, were measured in duplicate at 37 °C with an I.L. pH/gas analyzer Model 113. The P 50 was then corrected to pH = 7.40 using the factor of SEVERINGHAUS[12]. By this method, the average difference between 2 consecutive determinations of P 50 on the same blood was 0.3 ± 0.2 mm Hg.

Hct was measured in quadruplicate using Clay Adams micro-hematocrit tubes, and total Hb was measured in duplicate by the cyanmethemoglobin method. HbF was

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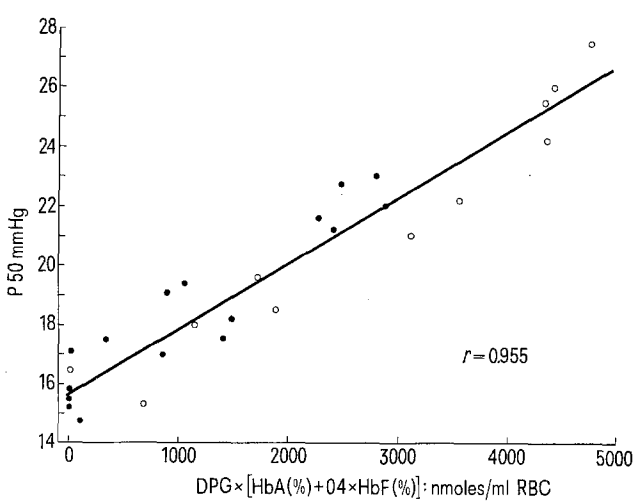

Fig. 1. Values of P 50 and of the function DPG × [HbA + β/α HbF] in adult (○) and fetal (●) blood stored at room temperature for various periods of time. Regression equation: P 50 = 0.00220 × DPG × [HbA + 0.4 HbF] + 15.69. $n = 27$; $r = 0.955$; $p < 0.001$.

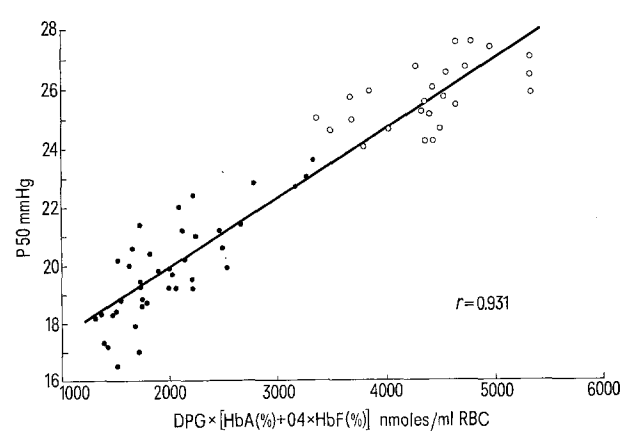

Fig. 2. Values of P 50 and of the function DPG × [HbA + β/α HbF] in fresh adult (●) and fetal (+) blood. Regression equation: P 50 = 0.00235 × DPG × [HbA + 0.4 HbF] + 15.24. $n = 65$; $r = 0.931$; $p < 0.001$.

determined in duplicate by the technique described by BRINKMAN and JONXIS[13]. The measurements of DPG were performed using the enzymatic method of TOWNE et al[14].

For the analysis of the results, it was assumed that the O_2 affinity of whole blood depends on the level of DPG, on the relative proportions of HbA and HbF and on the relative effect of DPG on the O_2 affinity of HbA and HbF. The relation among these variables can then be expressed by the following equation:

$$P50 = m \times [(\alpha \times DPG \times HbA) + (\beta \times DPG \times HbF)] + c \quad (1)$$

where α and β represent the relative effect of DPG on the P 50 of HBA and HbF, respectively; P 50 is expressed in mm Hg, DPG in nmoles/ml RBC and HbA and HbF as percent of total Hb; m and c are arbitrary factors representing the slope and the intercept of the regression line.

Equation No. 1 can also be written as follows:

$$P50 = m \times \alpha \times DPG\,[HbA + \beta/\alpha\, HbF] + c \quad (2)$$

where the the ratio β/α represents the effect of DPG on the P 50 of HbF in relation to the effect of DPG on the P 50 of HbA.

Regression equations were calculated for different values of β/α (0.1 to 1.0), for each group of experiments. The equations with the best fit, as judged by the correlation coefficients, were used to express the results and are shown in Figures 1 and 2.

The results in the two groups of experiments were very similar. There were no significant differences between the slopes or the intercepts of the two equations and in both cases the best correlation was obtained with a value of $\beta/\alpha = 0.4$. This suggests that in whole blood, both in vitro as well as in vivo, the effect of DPG on the P 50 of HbF is 40% of that of HbA. This value is in excellent agreement with those found by previous investigators using hemoglobin solutions[4, 5, 15].

Riassunto. Experimenti condotti sul sangue intero di individui neonati e adulti hanno dimostrato che l'affinità del sangue per l'ossigeno (P 50) dipende dalla concentrazione intraeritrocitaria di 2,3-difosfoglicerato (DPG) e dalle proporzioni relative di emoglobina adulta (HbA) e fetale (HbF). I risultati ottenuti indicano che nel sangue in toto l'effetto del DPG sulla P 50 della HbF è circa il 40% di quello sulla P 50 della HbA.

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Intracellular pH of Red Cells Stored in Acid Citrate Dextrose Medium

The importance of hydrogen ion cencentration during the storage of red cells has been noted and actually the controlled lowering of pH by citrate buffer was the major improvement in blood preservation. More recently, the levels of ATP and 2,3-diphosphoglycerate, which play an important role in the cells, have been shown to be greatly affected by the pH of the storage medium[1, 2]. Although there have been several reports on the pH change during storage, the measurements were done at 37°C[2] and the data obtained were extracellular pH (pH_e). It seems more interesting to know the intracellular pH (pH_i) of red cells if we aim to improve storage conditions, especially so in relation to the stability of intracellular enzymes or metabolic intermediates during storage. Moreover, the pH at the temperature of storage, i.e. at 4°C, may have more meaning for this end than the pH at body temperature. The present investigation is on the changes of the pH_e and the pH_i at 4°C of ACD blood during storage.

Methods. The pH_i was measured by using 5,5-dimethyl oxazolidine-2,4-dione (DMO) essentially according to the method of CALVEY[3]. To 100 ml of acid citrate dextrose (ACD) blood in a storage bottle, about 4 µCi of 2-C^{14} DMO (New England Nucl. Corp., spec. act. 10.1 mCi/m*M*) was added with a syringe and the blood was preserved at 4°C. Every 2 or 3 days, an aliquot of the sample (about 5 5 ml) was taken out by a syringe and used for the assay. The pH of the suspension (pH_e) was measured by a Hitachi-Horiba expanded scale pH-meter F-5 at 4°C. The samples were taken out before and after centrifugation (5,000 rpm for 10 min in a refrigerated centrifuge), using a precalibrated micropipette (99.8 µl). DMO was extracted from each sample and measured by a liquid scintillation spectrometer. The water content of the suspension was measured by drying the sample in a hot air oven at 110 ± 10°C for 24 h. The content of extracellular water in packed cells was measured by C^{14}-inulin for another batch of ACD blood and found to be 2 to 4% of packed cell volume, which showed no appreciable change during storage and contributed to the calculation of pH_i to a negligible extent. The pH_i was calculated according to the equation derived by IRVINE et al.[4], except that the measured value at 4°C of pK' = 6.52 was used for DMO.

Results and discussion. Typical data of ACD blood stored for 1 month are shown in the Table. The pH_i decreased as the pH_e of the blood decreased during the storage. The pH_i was always higher than the pH_e and the decrease of the pH_i during the storage was slower than that of the pH_e. The pH_e of ACD blood shown here is appreciably higher than that reported by other workers[2] because of the measurement at 4°C. The higher value of the pH at 4°C can be explained by big negative temperature coefficient ($\Delta pH/\Delta t$) of protein solution as a buffer system. For example, the pH of 2-day-old ACD blood was 7.37 at 4°C and 6.92 at 37°C. The pH of ACD plasma was affected by temperature to lesser extent than that of the suspension, e.g. 7.35 at 4°C and 7.08 at 37°C, indicating that the pH_i is affected by temperature change more than the pH_e.

It has been known that the pH_i of red cells is lower than the pH_e of fresh blood or the cells suspended in

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