2,3-Diphosphoglycerate Content and Oxygen Affinity as a Function of Red Cell Age in Normal Individuals

By STAVROS HAIDAS, DOMINIQUE LABIE, and JEAN-CLAUDE KAPLAN

A parallel decline of 2,3-diphosphoglycerate (2,3-DPG) and \( P_{50} \) of intracorpuscular hemoglobin is found in red blood cells during their in vivo aging. After 2,3-DPG depletion due to in vitro storage, the capacity to restore, 2,3-DPG in the presence of inosine is significantly impaired in senescent cells as compared with young cells.

The effect of 2,3-diphosphoglycerate (2,3-DPG) upon the oxygen affinity of hemoglobin\(^{1-5}\) and consequently upon the oxygen delivery of red cells to tissues is now well established.\(^{4,6}\)

It has been found that a shift to the left of the oxygen dissociation curve of intracorpuscular hemoglobin occurs in ACD blood during storage,\(^{7}\) and also in vivo in old red blood cells.\(^{8}\) More recently a decline of 2,3-DPG was also described in vitro and in vivo aged red blood cells.\(^{9}\)

However the simultaneous study of both 2,3-DPG and oxygen affinity in red blood cell fractions of different age has not previously been reported.

We present here our results of such a study. Furthermore, the capacity of depleted red cells to restore 2,3-DPG upon incubation with inosine was investigated, and found to be significantly different in in vivo aged red blood cells as compared with in vitro aged cells.

Materials and Methods

Twenty-four ml of heparinized venous blood was obtained from normal adults of both sexes working in the laboratory. Separation of red blood cells into different age fractions was performed by centrifugation in mixtures of phthalate esters of different specific gravity according to the method described by Danon et al.\(^{10,11}\)

After removal of leukocytes and reticulocytes in a preliminary step, four fractions were prepared: (1) Light or "young" red blood cells corresponding to the top fraction (10% of total). (2) Dense or "old" red blood cells corresponding to the bottom fraction (10% of total). (3) Intermediate red blood cells corresponding to the middle fraction (20% of total). The light fraction contained less than 1.5% reticulocytes. The other fractions were devoid of reticulocytes. (4) Total samples of unfractioned red blood cells were also...
Our results are expressed as μmoles 2,3-DPG per g Hb. If expressed per ml RBC the difference of 2,3-DPG between "old" and "young" cells would be lessened, but still significant.

Oxygen affinity

\[ P_{50} \]

\[ O_2 \] partial pressure (mm Hg)

2,3-DPG

\[ \mu \text{moles} \ 2,3\text{-DPG} / \text{g Hb} \]

submitted to the same stress as the other fractions by passing whole blood through a phthalate ester mixture.

All the red blood cell fractions (total, light, dense, and intermediate) were then thoroughly washed in saline to remove any contaminating phthalate esters. The quality of the separation was verified by measuring the activity of glucose-6-phosphate dehydrogenase (G6PD) and hexokinase (HK) in light and dense fractions. G6PD activity was 1.5 and HK activity 2.5-fold greater in the light fractions.

Oxygen equilibrium curves were determined spectrophotometrically in a Unicam SP 800 spectrophotometer, according to the technique of Benesch et al.\textsuperscript{12} modified by Bellingham and Huehns.\textsuperscript{13} Measurements were performed on washed red blood cell fractions suspended in isotonic phosphate buffer pH 7.13.

2,3-DPG was assayed according to Beutler’s modification of Krimsky’s method.\textsuperscript{14}

\[ \text{RESULTS AND DISCUSSION} \]

2,3-DPG and Oxygen Affinity in Red Blood Cell Fractions of Different Age

Determination of 2,3-DPG in young, intermediate, and old red blood cell fractions obtained in 10 individuals showed that aging of the cell is followed by a regular decline of 2,3-DPG (Fig. 1 and Table 1). Each category fell in a rather narrow range of 2,3-DPG level (Table 1). An average of 60% decrease of 2,3-DPG was found in the old cells as compared to the young fraction.*

The oxygen affinity of the different red blood cell fractions showed a regular decrease of \( P_{50} \) during the process of aging (Fig. 1 and Table 1).

The strict parallelism between the evolution of 2,3-DPG and \( P_{50} \) in young, intermediate and old red blood cells is clearly seen in Fig. 1.

*Our results are expressed as μmoles 2,3-DPG per g Hb. If expressed per ml RBC the difference of 2,3-DPG between "old" and "young" cells would be lessened, but still significant.
These data confirm the observations made by Edwards and Rigas on oxygen affinity,8 and Bunn and co-workers on 2,3-DPG.9 However, the difference of 2,3-DPG content between young and old red cells was more pronounced in our experiments. This is probably due to the greater efficiency of the phthalate esters method of cell separation as compared to the ultracentrifugation method. In contrast, Hjelm8 using the latter method found no difference in 2,3-DPG content of “young” and “old” red cells. Unexpectedly, the values for the P50 and 2,3-DPG of the total samples are nearer the values of the youngest cells instead of the intermediate fraction. Experimental errors due to some phthalate effect are not likely since all fractions including the total samples were exposed to phthalate.

The same results were obtained in “total” red cells not exposed to phthalate. It is possible that in the stepwise procedure of centrifugation we used, the youngest cells were lost along with the reticulocytes. Therefore only the densest (old) fraction could be considered pure enough, the others being less homogenous.

### 2,3-DPG Restoring Capacity of In Vitro and In Vivo Aged Red Blood Cells

During in vitro storage of blood in the usual conditions (ACD at 4°C) the level of red cell 2,3-DPG declines rapidly.9 It has been shown that after incubation with inosine the cells regain most of their original 2,3-DPG content.9,15 In vivo repletion of 2,3-DPG was also observed after reinfusion of depleted stored cells.16,17

We have compared the 2,3-DPG regenerating capacity of stored red cells of different in vivo age. Fresh blood drawn from two normal individuals was fractioned with phthalate mixtures into young and old red blood cells. A sham total fraction was also prepared as described under methods. 2,3-DPG was depleted by storing the different fractions at 4°C in saline without any exogenous source of energy for 13 days. At intervals, the 2,3-DPG content was measured in aliquots, before and after incubation for 1 hr at 37°C in saline containing 0.01 M sodium phosphate buffer pH 7.4, and 15 mM inosine.

From the results shown in Fig. 2 it is obvious that the decline of 2,3-DPG

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Fig. 2.—Capacity of total (T), young (Y) and old (O) red cells to restore 2,3-DPG in the presence of inosine before and after preliminary storage (see details in text).

is more pronounced in the older cells on the 13th day and that these cells have almost completely lost their capacity to replenish their 2,3-DPG content.

In contrast, young cells retain most of their 2,3-DPG restoring capacity throughout 2 wk of storage. This phenomenon is particularly striking if one compares the increment observed in young cells on the 13th day to that obtained with old cells on the 5th day, since both fractions have a 2,3-DPG level of the same range before incubation with inosine.

These findings suggest that the decrease of 2,3-DPG observed in stored red blood cells is intrinsically different from that observed in in vivo aged red blood cells. The in vitro loss of 2,3-DPG might merely reflect the depletion of energy rich compounds, whereas the in vivo phenomenon would be due rather to the impairment of 2,3-DPG synthesis in the senescent cells, since the capacity of hemoglobin to bind 2,3-DPG remains unaltered in old cells. The question still remains as to whether the metabolic handicap in these cells is located on the pathway leading from inosine to the main glycolytic route, or between triose phosphates and 2,3-DPG.

References

2. Benesch, R., and Benesch, R. E.: The effect of organic phosphates from the human
2,3-DPG CONTENT, OXYGEN AFFINITY, AND RED CELL AGE


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