FACTORS AFFECTING THE CONCENTRATION OF 2,3-DIPHOSPHOGLYCERATE IN ERYTHROCYTES

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Received 24 March 1972

1. Introduction

2,3-Diphosphoglycerate (2,3-DPG) is the major organic phosphate of human erythrocytes. Its role as regulator of the oxygenation properties of haemoglobin, due to its direct and specific binding with the latter, is well known [1–5]. The decrease in the level of 2,3-DPG and other intraerythrocytic phosphocompounds in blood stored for transfusion is also well known [6, 7]. Consequently, many studies have been carried out with the object of improving our knowledge of the factors (whether physiological or not) which regulate the amount of this phosphate in human red cells under these conditions [8–10].

In this communication we present a comparative study of some factors which affect the concentration of 2,3-DPG. We deal on the one hand with two sulphur compounds, tetrathionate and dithionite, oxidizing and reducing agents, respectively, which act upon the enzymes of the Rapoport Cycle [11], catalysts of the synthesis (2,3-DPG-mutase; EC 2.7.5.4) or of the degradation (2,3-DPG-phosphatase; EC 3.1.3.13) of 2,3-DPG [9, 10]. Our experiments show that tetrathionate maintains the levels of 2,3-DPG in stored blood by inhibiting the 2,3-DPG-phosphatase, while dithionite has the contrary effect. On the other hand, we deal with a pyrimido-pyrimidine derivative (dipyridamole) which has not only a well-defined pharmacological action (favouring the flow of oxygen to the myocardium), but also maintains the levels of endogenous 2,3-DPG [10]. Experiments with dipyridamole show that this compound maintains the level of 2,3-DPG in stored blood without affecting the activity of these two enzymes. The results have a possible practical application in maintaining the 2,3-DPG level in blood for transfusion. An analogous role has been suggested for dipyridamole as an agent preserving the levels of ATP [12].

2. Materials and methods

The experiments were carried out with 20 ml of heparinized blood from a single donor. 4 ml samples were added to sterilized tubes containing 1 ml of a solution of 0.07 M citrate and 0.14 M glucose, pH 5.0 (ACD, formula A, NIH). Two of these tubes also included 5 mM tetrathionate or dithionite by adding 0.25 ml of 100 mM sodium tetrathionate (K. and K. Laboratories, USA) or 100 mM sodium dithionite (BDH, England). A third sample included 0.5 mM dipyridamole by adding 0.0625 ml of 40 mM dipyridamole hydrochloride (Boehringer and Soehne, Ingelheim, Germany). The last sample, added to 0.25 ml of physiological saline, served as control. The final pH in all the samples was 7.0. The mixtures were maintained at 4° and aliquots were withdrawn and used for the estimation of 2,3-DPG levels and for the assay of enzyme activities at zero time and at weekly intervals.

2,3-DPG was determined by Bartlett's method [13] using chromotropic acid (Merck, Germany) adapted to samples of blood by Eaton et al. [14]. On some occasions the enzymatic method of Towne et al. [15] was employed. The activity of the 2,3-DPG-phosphatase was measured using the method developed by Harkness et al. [16], and that of 2,3-DPG-mutase according to method A of Joyce et al. [17]. The preparation of 3-phosphoglycerate free from 2,3-DPG necessary for the measurement of the activity of the

mutase, was carried out according to Grisolia et al. [18].

3. Results and discussion

As can be seen in fig. 1, the level of 2,3-DPG in human blood is better maintained during storage in the presence of tetrathionate. Thus, in about 21 days, the 2,3-DPG level fell to 50% of its initial value in the control but to only 90% in the presence of tetrathionate. Fig. 2 shows the results obtained with rabbit blood treated under identical conditions; in this case the effect of tetrathionate is also evident from the beginning of the period of conservation. The effects of dithionite and dipyridamole are shown in fig. 3, where treatment with tetrathionate is also included for comparison. Dipyridamole maintains the 2,3-DPG levels while dithionite has the opposite effect.

The variation of 2,3-DPG levels under the influence of these three compounds could be due to direct action upon the enzymes for its synthesis and degradation. The activity of 2,3-DPG-phosphatase (fig. 4) in ACD human blood is progressively inhibited by tetrathionate, and activated by dithionite but dipyridamole does not alter the activity of this enzyme. The values for 2,3-DPG-mutase (data not shown) indicate only a slight inhibition in the presence of tetrathionate, dipyridamole and dithionite having no significant effect. Furthermore, both enzymatic activities remained constant in the control blood throughout the period of storage.

Thus the preservation of 2,3-DPG levels by tetrathionate (figs. 1–2) appears to be due to the decreased activity of the enzyme which degrades it (2,3-DPG-phosphatase; fig. 4). The results confirm those obtained by Gerlach et al. [10] and might be explained by specific oxidation of –SH groups as has been shown to occur in other enzyme systems [19].

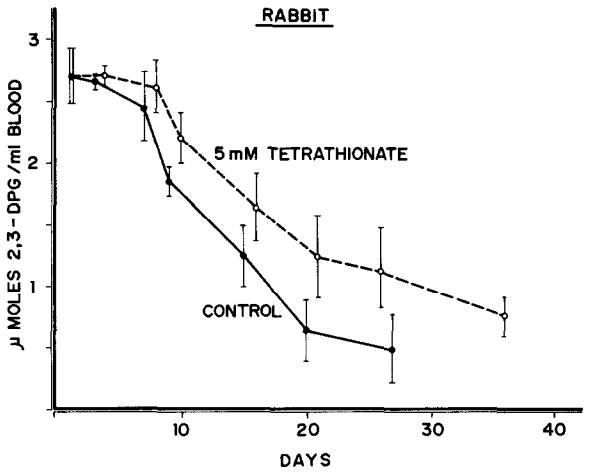

Fig. 2. Effect of tetrathionate on the 2,3-DPG levels of ACD rabbit blood stored at 4°. Experimental conditions are described in the text.

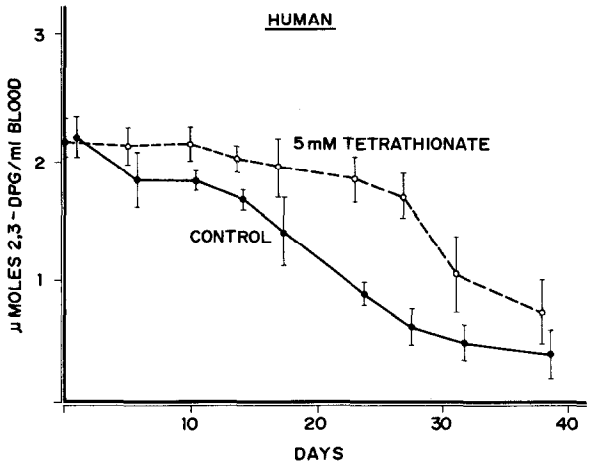

Fig. 1. Effect of tetrathionate on the 2,3-DPG levels of ACD human blood stored at 4°. Experimental conditions are described in the text.

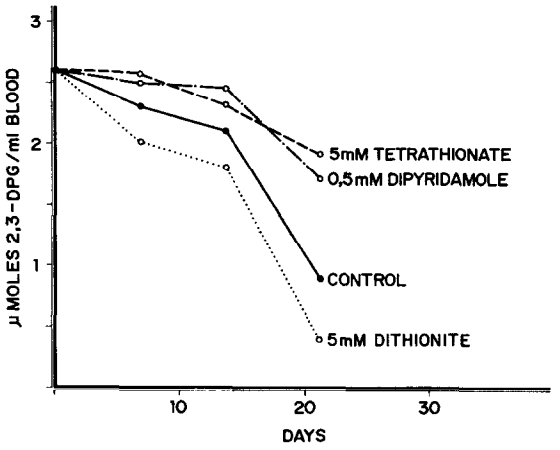

Fig. 3. Comparative effect of dipyridamole and sulphur compounds on the 2,3-DPG levels of ACD human blood stored at 4°. Experimental conditions are described in the text.

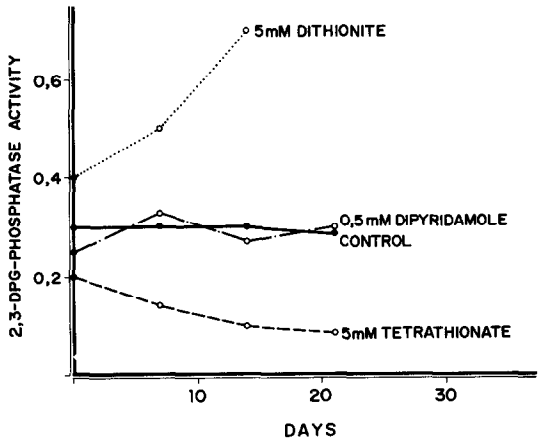

Fig. 4. The influence of dipyridamole and sulphur compounds on the 2,3-DPG-phosphatase activity of ACD human blood stored at 4°. The results are expressed in micromoles of 2,3-DPG hydrolyzed by 0.5 ml of whole blood hemolysate during a 2 hr period of incubation at 38°, pH 7.0, under conditions described by Harkness et al. [16].

Dithionite, a strong reducing agent, stimulates 2,3-DPG-phosphatase (fig. 4) presumably by keeping reduced a group needed for its activity (probably –SH). This would explain the decrease in the levels of 2,3-DPG under its influence (fig. 3).

Dipyridamole does not act directly upon these enzymes, but, nevertheless it also maintains the 2,3-DPG at a high level (fig. 3); we must, therefore, suppose the existence of an indirect mechanism. It has been suggested [10] that dipyridamole induces a fall in the concentration of ADP by reducing the permeability of erythrocytes for adenosine, thus causing a rise of 1,3-diphosphoglycerate (1,3-DPG), via phosphoglycerate kinase, and therefore of 2,3-DPG, via 2,3-DPG-mutase.

In summary the results presented here are consistent, firstly, with the hypothesis of the "*in vivo*" regulation of 2,3-DPG by means of ADP [10, 20]; secondly, indicate that some reduced –SH groups are necessary for the activity of 2,3-DPG-phosphatase; and lastly, the use of dipyridamole may be useful in improving the viability of blood stored for transfusion.

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