Stimulative Effect of 2,3-Diphosphoglycerate on Methemoglobin Reduction by Ascorbic Acid

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Summary. The reduction of methemoglobin by ascorbic acid was accelerated by the presence of 2, 3-diphosphoglycerate.

The physiological role of 2, 3-diphosphoglycerate (2, 3-DPG), which is the most abundant phosphorus compound in red cells, has been clarified since the discovery by BENESCH et al.[1] and CHANUTIN and CURNISH[2] that it lowers the oxygen affinity of hemoglobin and thus facilitates the release of oxygen. Lately DUHM[3,4] showed that organic phosphates, especially 2, 3-DPG, regulate intracellular pH as participants of the Donnan Gibbs' equilibrium. On the other hand, BEUTLER et al.[5] indicated the inhibitory effect of 2, 3-DPG on glycolytic enzymes. This paper deals with another effect of this phosphorus compound, i.e. the stimulative effect on methemoglobin reduction in the presence of ascorbic acid.

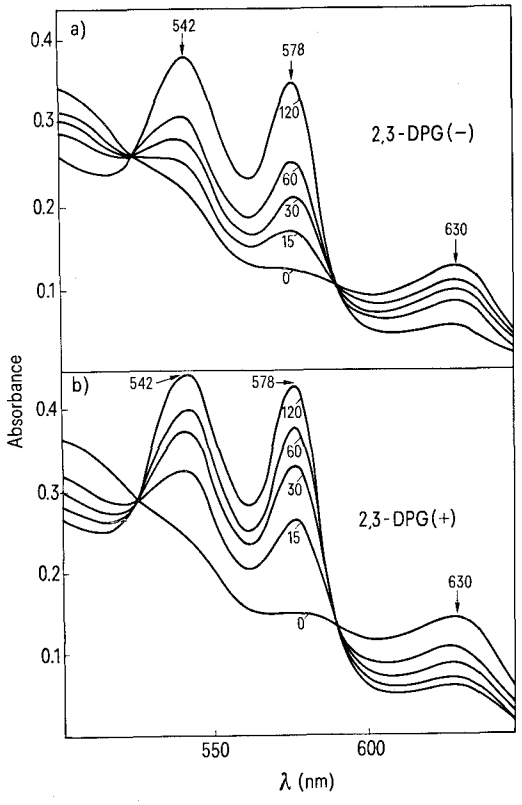

Fig. 1. Spectral change of methemoglobin to oxyhemoglobin between 500 nm and 650 nm during a 2-hour period after the addition of ascorbic acid. a) In the absence of 2,3-DPG. b) In the presence of 2,3-DPG (final concentration 2.6 m*M*).

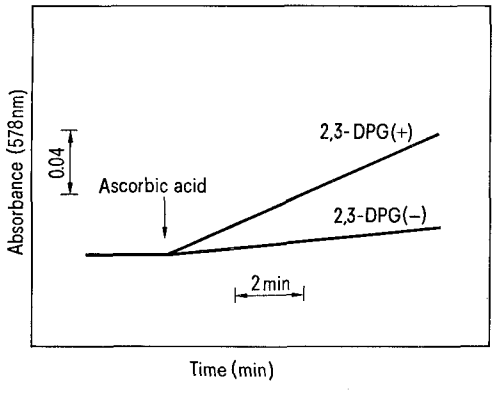

Fig. 2. Rate of methemoglobin reduction in the absence and presence of 2,3-DPG (final concentration 2.6 m*M*) after the addition of ascorbic acid (final concentration 6 m*M*).

Although high concentration of 2, 3-DPG in red cells is expected to exert some influence on the redox reaction of hemoglobin, there are few reports about the effect of this compound on the methemoglobin reduction. We explored the influence of this phosphorus compound on the methemoglobin reduction initiated by the addition of ascorbic acid, which reacts with methemoglobin directly without being affected by the enzymatic methemoglobin reducing activity in red cells[6].

Methods. Hemoglobin A, obtained from fresh human red cells by hemolysis, was converted to methemoglobin by the addition of potassium ferricyanide. After removal of ferricyanide with Dowex I-8, methemoglobin was desalted by passing through a Sephadex G-25 column previously equilibrated with 0.05 *M bis-tris* buffer (pH 7.0) containing 0.1 *M* NaCl. Experiments were performed as follows. $^1/_{10}$ ml of 0.05 *M bis-tris* buffer (pH 7.0) containing 0.1 *M* NaCl and 1.4 ml of methemoglobin (final concentration 12 μ*M*) were mixed with or without 2,3-DPG. After mixing and 5 min standing, the reaction was started by the addition of 20 μl of 450 m*M* sodium ascorbate solution and the rate of methemoglobin reduction was spectrophotometrically measured at 25 °C by following the increase in absorbance at 578 nm.

Results and discussion. Figure 1a and b shows the change in the spectra of methemoglobin between 500 nm and 650 nm during a 2-hour period after the addition of ascorbic acid. Relatively slow reduction of methemoglobin to oxyhemoglobin occurred by the addition of ascorbic acid, which is characterized by the increase in the absorbance at 542, 578 nm and the decrease at 630 nm. Obviously 2, 3-DPG accelerated these spectral changes of methemoglobin to oxyhemoglobin by ascorbic acid. As the acceleration by 2, 3-DPG was noticed, the initial velocity of methemoglobin reduction was measured by following the increase in the absorbance at 578 nm (Figure 2). A 4 times increase in rate was observed in the presence of 2, 3-DPG compared with the control without 2, 3-DPG.

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It is known that inositol hexaphosphate (IHP), the stronger effector, changes the redox potential of ferricferrous hemoglobin[7] and also changes the conformation of methemoglobin[8]. Since 2,3-DPG, though weaker than IHP as an effector, is also shown to change the redox potential of the same system[9], it is likely that the conformation of methemoglobin is changed by 2,3-DPG. A plausible explanation for the acceleration may be attributed to the increase in reactivity of ascorbic acid with methemoglobin, which is facilitated by the change of conformation of the latter due to the binding of 2,3-DPG. However, detailed mechanism of the reaction remains to be clarified.

These results suggest that 2,3-DPG affects the rate of methemoglobin reduction in intact red cells, especially when ascorbic acid is applied to patients suffering from hereditary methemoglobinemia. This is consistent with the report[10] that the highly increased content of 2,3-DPG in red cells of hereditary methemoglobinemia.

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The Effect of Renal Hydrodynamics on Immune Complex Deposition[1]

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Summary. The role of renal hydrodynamics on renal deposition of immune complexes was evaluated in acute serum sickness. Using i.v. radiolabelled antigen in rabbits under a variety of hydrodynamic alterations, these studies suggested that although intrarenal hydrodynamics influence renal deposition of immune complexes factors other than intrarenal hydrostatic pressure may be important.

It is well recognized that circulating antigen-antibody immune complexes deposit in tissues, particularly vascular membranes, resulting in an inflammatory injury through activation of complement[4]. Immune complex glomerulonephritis has been studied extensively by means of the acute serum sickness model[5]; however the effedts of renal hydrodynamics on immune complex deposition have not been well defined. GERMUTH et al.[6] reported that renal hydrodynamics were a determinant of the site of deposition and injury caused by complexes. These investigators suggested that clamping of the renal artery or ligation of the ureter induced the morphological and immunohistological differences in the kidneys in serum sickness, and they postulated that these differences are secondary to the differences of immune complex deposition; however, they did not quantitate the amount of immune complexes which were deposited.

Materials and methods. Human serum albumin (HSA) was labelled with I^{125} by the chloramine-T methods as described by MCCONAHEY and DIXON[7]. Ten 3 kg rabbits were injected i.v. with I*HSA 250 mg/kg. On the same day 10 ml/kg HSA in Freund's complete adjuvant was injected s.c. to ensure an immune response. Blood samples were taken at frequent intervals from the central ear artery using butterfly scalp vein needles and aseptic technique. Blood was allowed to clot at 37 °C for 2 h. The clotted blood was centrifuged at 2,000 rpm and the serum was decanted. Protein from 1 ml of serum was precipitated in 10% trichloracetic acid (TCA), washed by centrifugation and counted to determine total serum I*HSA.I*HSA complexed with antibody was determined by the ammonium sulfate precipitation technique of FARR[8] using fresh non-refrigerated serum. 2 days after administration of antigen the animals were divided into 5 groups with 2 animals in each group. Group I: controls which received no hydrodynamic manipulations. Group II: the right renal vein was ligated. Group III: the left renal vein was ligated. Group IV: the right ureter was ligated. Group V: the left ureter was ligated. Blood was obtained for detection of I*HSA or I*HSA immune complexes. The animals were sacrificed on the 16th day, at which time the kidneys were removed and studied by light and immunofluorescent microscopy after their radioactivity had been measured. The method of immunofluorescent microscopy has been described previously[9].

Results. Free I*HSA was detected in the serum until day 8. Following this time I*HSA immune complexes were detected in the serum. On the 15th day, the day prior to sacrifice, no free I*HSA or I*HSA immune complexes were detected in the serum.

Deposits of rabbit IgG, C3, and HSA were detected in both kidneys of all animals. Kidneys subjected to ureteral ligation were hydronephrotic whereas those subjected to renal vein ligation were sclerotic.

Discussion. Renal hydrodynamic forces have been suggested to play a role in deposition of circulating immune complexes[10]. Increase arterial blood pressure pre-

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