THE EFFECT OF ORGANIC PHOSPHATES FROM THE HUMAN ERYTHROCYTE ON THE ALLOSTERIC PROPERTIES OF HEMOGLOBIN

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It has been known for a long time that the characteristic sigmoid oxygen dissociation curve of hemoglobin is obtained only in the presence of certain salts (Barcroft & Camis, 1909; Barcroft & Roberts, 1909). This influence of electrolytes on ligand binding has recently been reinvestigated by Rossi-Fanelli <u>et al</u>. (1961) and Enoki & Tyuma (1964) with results which confirm the effect of many of these ions both on the position and the shape of the oxygenation curve.

We have now investigated the effect of some of the organic phosphate esters which form the normal environment of hemoglobin in the red blood cell and have found that some of these compounds have a profound influence on the oxygenation of hemoglobin at the concentrations in which they occur in the erythrocyte.

Human hemoglobin was prepared as described previously (Benesch & Benesch, 1961) and the oxygen dissociation curves were determined by the method of Benesch <u>et al</u>.

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(1965) on 0.3% solutions of hemoglobin.

As can be seen from Fig. 1, the oxygenation curve of dialysed hemoglobin in the absence of added electrolytes shows no significant heme-heme interaction (n* = 1.1) and the oxygen affinity is very high (log p_{50}** = 0.16). This is in agreement with previous work (Rossi-Fanelli <u>et al.</u> 1961, Enoki & Tyuma 1964). Neither NaCl nor Na_2SO_4 had a measurable influence on these parameters at an ionic strength of 0.01. Phosphate, on the other hand, shows a distinct effect at this ionic strength (n = 1.9, log p_{50} = 0.40). A dramatic effect, however, is shown by 2,3-DPG*** which increases heme-heme interaction to the

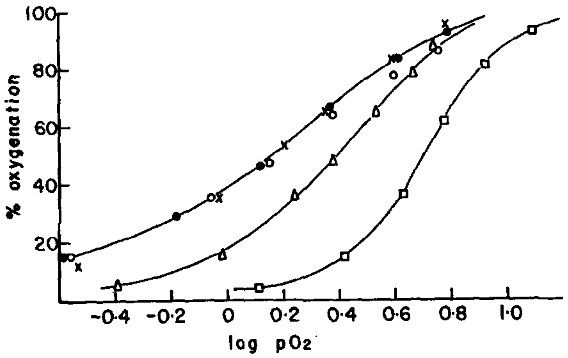

Fig. 1: The effect of salts on the oxygenation of hemoglobin

●—● 0.3% hemoglobin in water
○—○ " " " 0.01 M NaCl; $\mu = 0.01$
×—× " " " 0.0033 M Na_2SO_4; $\mu = 0.01$
△—△ " " " 0.0045 M potassium phosphate; $\mu = 0.01$
□—□ " " " 0.0014 M 2,3-DPG; $\mu = 0.01$

Temperature = 10°C, pH = 7.0

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* n is the exponent in the Hill equation: $(HbO_2)/(Hb) = K(pO_2)^n$
** p_{50} is the oxygen pressure at 50% oxygenation
*** 2,3-diphosphoglyceric acid

maximum observed under physiological conditions ($n = 2.7$) and decreases the oxygen affinity to normal values ($\log p_{50} = 0.72$). Since this comparison was made at constant ionic strength, the molar concentration of 2,3-DPG was only 1/7 that of the NaCl or about 1/3 that of the inorganic phosphate.

The oxygen affinity ($\log p_{50}$) and the degree of heme-heme interaction of hemoglobin is shown as a function of the molar concentration of 2,3-DPG in Fig. 2. It is clear that both these properties have reached their maximum value at the concentrations of this compound reported in the erythrocyte (Bishop, 1964). Similar results to those shown in Figs. 1 and 2 were also obtained at 30°C. A comparison of the oxygenation curves of hemoglobin in 2×10^{-3} M 2,3-DPG at this temperature with those obtained by Astrup et al (1965) on whole human blood shows an extremely close agreement between the two curves (Table 1).

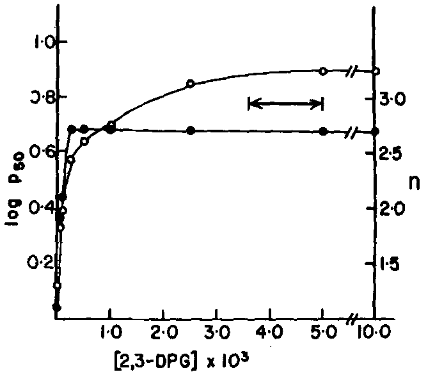

Fig. 2: Dependence of $\log p_{50}$ and n on the concentration of 2,3-DPG

o——o = $\log p_{50}$

●——● = n

|←——→| = range of concentration of 2,3-DPG in normal human erythrocytes

TABLE I

Oxygen Affinity, Heme-Heme Interaction
and Bohr Effect at 30°C

	Whole Blood (Astrup et al 1965)	Hb in 2 x 10^{-3}M 2,3-DPG
Log p_{50} pH 6.94	1.45*	1.46
Log p_{50} pH 7.69	1.07*	1.04
n	2.7	2.7
Δ log p_{50}/Δ pH	0.50	0.56

* Calculated from the values at pH 7.40 and Δ log p_{50}/Δ pH

The effects of other organic phosphates on the oxygenation behavior of hemoglobin together with their concentration in human erythrocytes are shown in Table II. It is clear that they fall into the three categories shown, inorganic phosphate being the least effective, followed by the monophosphates, while the di and triphosphates are by far the most potent. Among these only 2,3-DPG and ATP are present in substantial concentrations in the red cell.

The mechanism of the interaction of organic phosphates with hemoglobin is now under investigation, but more specific effects than charge alone are clearly involved. This is illustrated by the complete ineffectiveness of sulfate or the limited effectiveness of inorganic phosphate, as compared with DPG. Even ferrocyanide, which has a higher negative charge than 2,3-DPG at neutral pH, has only about as much influence as inorganic phosphate on cooperative ligand binding by hemoglobin.

Although it has been known since 1925 (Greenwald, 1925) that the red cell contains large quantities of 2,3-DPG, the physiological role of this compound has not been clarified.

TABLE II

Effect of Phosphate Esters on the Oxygenation of Hemoglobin

Compound	n	log P_{50}	Molar concentration in Erythrocyte x 10^3 (Bishop 1964)
None	1.1	0.12	
Inorganic phosphate	1.9	0.38	0.28-0.48
3-Phosphoglycerate	2.7	0.50	0.05-0.08
2-Phosphoglycerate	2.5	0.59	
Adenosine monophosphate	1.8	0.65	0.01-0.02
Pyrophosphate	2.7	0.73	--
Adenosine diphosphate	2.4	0.88	0.19-0.25
Adenosine triphosphate	2.5	0.84	0.9-1.2
2,3-Diphosphoglycerate	2.7	0.90	3.6-5.0

Temperature 10°C, pH = 7; concentration of phosphates = 5×10^{-3} M

The results reported here strongly suggest that 2,3-DPG can act as a powerful regulator of the allosteric properties of hemoglobin in the human red cell, thus enabling it to act as an oxygen donor under physiological conditions.

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