HEMOGLOBIN E: ITS OXYGEN AFFINITY IN RELATION WITH THE IONIC ENVIRONMENT

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1. Introduction

In hemoglobin E ($\alpha_2\beta_2$ 26 (B8) Glu → Lys), the structural abnormality is located in the contact area between unlike subunits of one $\alpha\beta$ dimer. According to the structure–function relationship of the molecule of hemoglobin described by Perutz and Lehmann [1], a disturbance of its function could be expected.

The first oxygen equilibrium studies performed on blood or on intact cells, suggested an abnormally low oxygen affinity [2] and a tentative explanation was proposed at the molecular level [3]. In 1972, the oxygen equilibrium of hemoglobin E was more thoroughly studied by Bunn et al. [4]: they found an identical oxygen equilibrium in phosphate-free hemolysates of blood from E and A homozygotes and in purified components from heterozygotes. The former studies had been performed before the regulatory function of 2,3 diphosphoglycerate (2,3 DPG) was well understood [5, 6]. It was suggested that the observed discrepancy could be explained by a variable increase of the intra-erythrocytic 2,3 DPG.

In this work, a more complete study of hemoglobin E was performed in individuals carrying various genetic associations. From the data obtained on whole blood, intact cells, phosphate-free lysates and purified components, evidence is brought for a normal oxygen equilibrium and a normal regulatory effect of the 2,3 DPG in physiological salt conditions but for an increased oxygen affinity at low salt concentration.

2. Materials and methods

Two siblings heterozygous for hemoglobin E and A as well as their father, double heterozygous for hemoglobin E and β^0 thalassemia (in which only hemoglobin E and F are present), were found in a French family. Blood was also obtained from an Indonesian female homozygous for hemoglobin E. The main hematological data concerning these cases are summarized in table 1, a more detailed clinical study will be published elsewhere.

The hemolysates were prepared by routine procedure. The hemoglobins were isolated by DEAE-Sepha-

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Table 1
Main hematological data concerning the studied patients

Samples	RBC $10^6/mm^3$	Hb g/100 ml	PCV %	MCV $\mu\mu^3$	MCHC %	Hb
Fam. F.						
Case II-1 (father)	3.4	8.2	28	82.5	29	E + F (F = 40%)
Case III-1	5.9	14.1	43.5	73	32	E/A (F = 0%)
Case III-2	5.6	12.4	40	71	36	E/A (F = 1%)
Mrs P.	6.5	12.5	38	58.5	33	E/E

dex chromatography [7] and hemoglobin E was identified in each case by the amino acid analysis of the abnormal peptides, and sequence determination.

All the hemoglobin solutions were studied phosphate-free and prepared as described by May and Huehns [8] slightly modified.

The oxygen equilibrium was studied by the spectrophotometric technique of Benesch et al. [9] as modified by Bellingham and Huehns [2] on whole cells, phosphate-free hemolysates and pure components. On lysates and pure hemoglobins, the measurements were done in bis–Tris 0.05 M, pH 7.10 buffer. On whole blood the oxygen affinity was studied by Duvelleroy on his own setting [10].

Th regulation by 2,3 DPG was studied by comparing the successive oxygen equilibrium curves after adding in the tonometer, prior to deoxygenation, known quantities of 2,3 DPG to the sample in the same bis–Tris buffer.

Concentration of 2,3 DPG was estimated according to Beutler's modification of Krimsky's method [11].

Table 2
2,3 DPG contents and oxygen equilibria of intact red blood cells suspended in isotonic phosphate buffer pH 7.13 at 37°C

Sample	2,3 DPG μmoles/g Hb	Oxygen equilibria P 50	Hill's coefficient
Controls (A/A)	14.7 ± 1.0	29.5 ± 0.5	2.7–3.0
Fam. F.			
Case II-1 (father: E+F)	17.0	31.5	2.9
	?	31	3.0
	15.0	29.5	3.0
Case III-1 (E/A)	15.5	29.5	2.8
Case III-2 (E/A)	14.8	30	2.8
Mrs P. (E/E)	22.1	36	2.8

3. Results and discussion

(1) The oxygen equilibrium was studied on intact red blood cells suspended in isotonic phosphate buffer pH 7.13 (table 2). Only the results of the homozygous E/E patient were apparently abnormal, the P 50 and the 2,3 DPG being both increased. Nevertheless the correlated values remained in the normal curvilinear statistical range [12]. The 'shift to the right' is in agreement with the previously described results [2] and can be easily explained by a compensatory mechanism, the starting point of which is not yet well known.

In the case of the patient with hemoglobin E/thalassemia^O disease, disorders due to the severe anemia were expected, but the results were repeatedly found in the normal range. They were confirmed by measurements made on whole blood, corrected to pH 7.40. The found values of the P 50 were normal for the heteroxygous subjects: 26.1 and 26.7 mm Hg (normal 26.6 Hg) and not significantly different from the normal in the case of the patient carrying hemoglobin E and hemoglobin F: 25.9 and 25.3 mm Hg. A more complex regulatory mechanism is likely to be in-

Table 3
Oxygen equilibria of phosphate-free lysates in bis–Tris 0.05 M pH 7.10 Buffer at 37°C

Sample	P 50	Hill's coefficient
A/A	7.1	2.8
E/E	6.6	2.7
E/A	7.1	2.3
E + F	7.4	2.3

volved in order to explain the almost normal final adjustement.

(2) The phosphate-free lysates were studied in 0.05 M bis–Tris buffer pH 7.10 at 37°C (table 3). The slight differences of the P 50 in the different mixtures were apparently clear but had to be confirmed by a more accurate study of the pure components. The

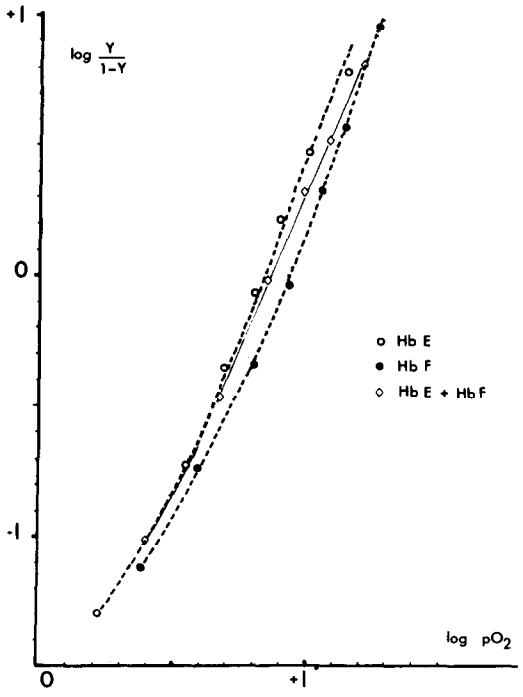

Fig. 1. Hill's plot of the oxygen affinity of phosphate-free lysates. The measurements were done at 37°C in bis–Tris 0.05 M pH 7.10 buffer on lysates 'stripped' by gel filtration on Sephadex G 25. The differences in the P 50 and in the value of the interaction coefficient appear on these curves. Similar results were obtained when comparing E/E, A/E and A/A lysates.

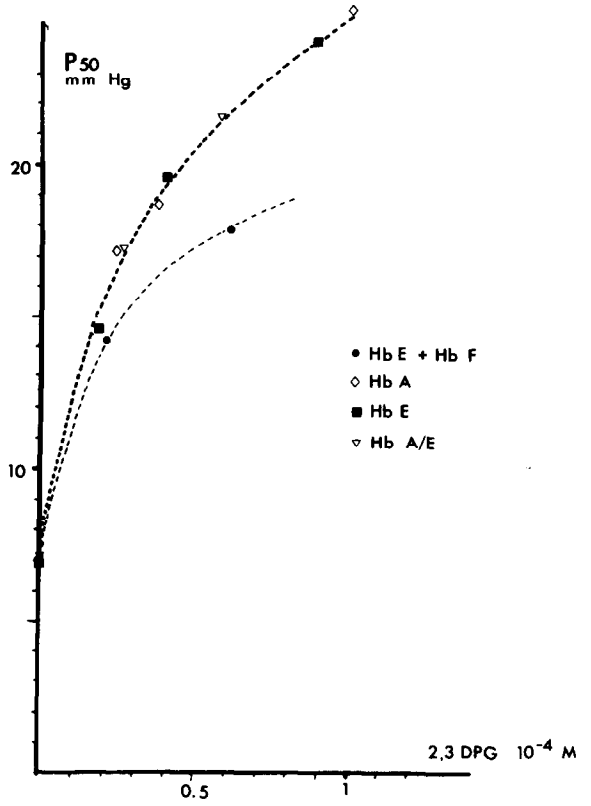

Fig. 2. Action of 2,3 DPG on 'stripped' lysates. The lysates were prepared in the way already described. The regulatory action of added 2,3 DPG is the same in the case of A/A, A/E and E/E lysates. It is decreased in the presence of a lysate containing 40% hemoglobin F.

Hill's coefficient was normal in lysates containing only one major component (A/A or E/E) and slightly decreased in mixtures of two different hemoglobins (A/E or E + F) (fig. 1). It is tempting to explain the intermediary curve as being the summation of two independent curves having the same interaction coefficient and a P 50 slightly different.

Known amounts of 2,3 DPG were added to the different lysates and its regulatory effect studied at concentrations ranging from 0 to 1×10^{-4} M (fig. 2). The action of 2,3 DPG was found identical for all the studied lysates with the exception of the one containing 40% hemoglobin F, where it was, as expected, decreased.

(3) The pure components were studied after DEAE-Sephadex chromatography and further Sephadex fil-

Table 4
Oxygen equilibria of pure components isolated by DEAE-Sephadex chromatography

Sample	P 50 mm Hg		Hill's coefficient	
	25°C	37°C	25°C	37°C
Hb A	2.9	7.2	2.6	2.8
Hb E	2.5	5.2	2.6	2.8
Hb F	3.6	8.3	3.0	2.7

tration in the same 0.05 M bis–Tris buffer. The oxygen equilibrium curves were determined at 37°C and at 25°C, and the oxidation checked not to exceed 5% at the end of the measurement (table 4). At both temperatures, a decrease of hemoglobin F and an increase of hemoglobin E oxygen affinity, as compared to hemoglobin A, were found approximately at the same ratio.

There is an apparent discrepancy between these results and the results published by Bunn working at a more physiological ionic strength [4]. The role of the saline environment is also confirmed by our normal results when working on intact cells or in the presence of 2,3 DPG. Nevertheless, when summarizing all our personal data, we can conclude for a slight increase of the oxygen affinity of hemoglobin E at low salt concentration. This small difference could be overlooked or disappear in the presence of added NaCl and be more evident in a low ionic strength buffer where the overall free energy of interaction among oxygen combining sites is lowered [13]. The newly introduced positive charge in the contact area may slightly destabilize the normal contact and decrease the free energy of interaction, therefore explaining the higher oxygen affinity.

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