THE EFFECT OF HEME AND IRON ON HAEMOGLOBIN A AND F SYNTHESIS IN HUMAN CORD BLOOD

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1. Introduction

Addition of heme to avian erythrocytes and rabbit reticulocytes stimulates globin synthesis. Hammel and Bessman [1] followed globin synthesis in nuclei of pigeon erythrocytes and proved its stimulation by heme and hypoxia. Bruns and London [2] found stimulated globin synthesis after the addition of heme in reticulocytes of rabbits which were chronically bled and simultaneously fed on iron deficient diet. The mechanism of this effect of heme is not yet completely clear. Heme can increase globin synthesis either by stabilization of polyribosomes, as has been suggested by Waxman and Rabinovitz [3], or by its attachment to completed globin chains which are released from ribosomes [4].

We have analysed the effect of heme on haemoglobin A and F synthesis in human cord blood. In our further experiments we studied the dependence of the stimulatory effect of heme on the concentration of iron in the incubation medium.

2. Methods

Experiments were carried out on human cord blood obtained at delivery. Incubation flasks contained 5 ml of packed cells in 50 ml of media. The incubation mixture included 30% of human plasma and of synthetic media (SEVAC Prague) containing all amino acids. Valine ^{14}C was used for the follow up of haemoglobin synthesis. Haemin was prepared according to Karibian and London [5] and added to the incubation mixture in the concentration of 1×10^{-4}M. To some incubation flasks iron was added in a concentration of 1×10^{-4}M. Haemoglobin F and haemoglobin A were prepared from human cord blood according to Zade-Oppen [6] with the use of CM-Sephadex 50 column. Globin was prepared from the eluate according to Rossi-Fanelli [7] by precipitation with acetone acidified with HCl at $-20°$C. When measuring the activity of haemoglobin A and F globin was dissolved in 0.2M formic acid and the sample was evaporated on aluminium discs. The protein content was determined in the dissolved sample after neutralization according to Lowry [8] and the activity was measured in a gas flow counter. The specific activity was expressed as cpm/mg globin.

3. Results and discussion

Table 1 shows stimulation of globin synthesis by heme added in the concentration 1×10^{-4}M to the incubation medium in reticulocytes of human cord blood. The degree of stimulation is not so considerable as in rabbit reticulocytes with an iron deficiency. There are no great differences in the stimulation of globin synthesis from A and F haemoglobins, though the stimulation of haemoglobin F synthesis was more pronounced in all our experiments. The greatest stimulation was found in the group to which, besides heme, iron in a concentration of 1×10^{-4}M was added. Also iron alone stimulates under used conditions the synthesis of both haemoglobins. Bruns and London found the greatest effect of haemin or of iron in increasing the formation of haemoglobin in cells derived

Table 1
The effect of iron and haemin on the synthesis of Hb A and Hb F in human cord blood. Iron was added as $FeSO_4(NH_4)_2SO_4 \cdot 6H_2O$.

	Hb A		Hb F	
	cpm/10 mg globin	%	cpm/10 mg globin	%
Control reticulocytes	422	100	97	100
Reticulocytes with 10^{-4}M Fe^{++}	480	114	112	115
Reticulocytes with 10^{-4}M haemin	505	120	121	125
Reticulocytes with 10^{-4}M haemin and 10^{-4}M Fe^{++}	557	132	139	143

from animals that had been rendered iron-deficient by diet and extensive phlebotomy. We therefore consider our findings as an indirect proof that reticulocytes of human cord blood are also iron-deficient.

Our results show that the stimulation of globin synthesis by heme can be demonstrated in human reticulocytes from cord blood. The degree of the stimulation depends on the amount of iron present in the incubation mixture.

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