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EFFECT OF IRON SUPPLEMENT ON Hb SYNTHESIS BY IRON DEFICIENT RETICULOCYTES – AN *IN VITRO* STUDY

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Abstract : Iron uptake for hemoglobin synthesis was apparently increased in the reticulocytes of severely iron deficient anemic women, but was actually decreased significantly when expressed in terms of number of reticulocytes. The decreased synthesis could be restored to more than normal by supplementing with ferric iron or transferrin iron (normal plasma), but not with ferrous iron, which rather further reduced the already impaired hemoglobin synthesis.

Key words : iron uptake, hemoglobin synthesis, iron deficiency anemia, reticulocytes

INTRODUCTION

Anemia is a condition in which the quality or quantity of circulating hemoglobin is reduced, and iron deficiency anemia is one in which the rate of hemoglobin synthesis is arrested by limiting amounts of available iron.

In vitro iron uptake for *de novo* hemoglobin synthesis by reticulocytes has been under investigation by various workers (1-4). However these studies were conducted under conditions far from physiological, and were confined to reticulocytes from non-anemic persons. Therefore, to know whether iron uptake and hemoglobin synthesis proceeds unabated at normal rate in iron deficiency anemia or are altered in the face of decreased iron availability, and if so, how to restore it to normal, is the subject matter of this investigation.

METHODS

Non-pregnant females attending outdoor of Mahila Chikitsalaya, Jaipur, for gynaecological problems, and who were free from any infection or febrile disease were requested to participate in the study. The women with hemoglobin 12g% or above were grouped in normal control (non-anemic) group and those having less than that in anemic group (5). Every effort was made to select anemic women with as severe anemia as possible.

Blood, collected in a heparinized vial, was immediately used for radioiron studies, as well as analysed for hematologic values

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(6), and plasma iron values by ferene chromogen on an auto analyzer (Merck, Selectra).

Radioiron studies : The radioactive experiments were designed basically as done by Ponka & Neuwirt (7).

Radioactive iron (Fe-59) was obtained from Bhabha Atomic Research Centre, Mumbai. The material contained 0.89 mCi activity as ferric chloride in HCl in a total volume, 1.5 ml. It was diluted by normal saline to get an activity of 1 μCi/ml.

Before studying *de novo* Hb synthesis, pilot experiments conducted to determine optimum dose of radioiron and time of incubation. One milliliter of freshly collected whole blood was incubated with varying activities of radioiron (0.2, 0.4, 0.6, 0.8 and 1.0 μCi) for specified time (15, 30, 45 and 60 minutes) at 37°C. It was found that the optimum dose of radioiron was 0.6 μCi(0.6 ml of diluted material) and optimum period of incubation was 30 minutes. The rate of Hb synthesis was linear during this time with this dose at 37°C. Thus the iron uptake was studied at physiological conditions with respect to temperature, pH and additives.

In all subsequent studies with control and anemic blood, 1.0 ml of blood was mixed thoroughly with 0.6 μCi(0.6 ml) of radioiron and incubated at 37°C for 30 minutes, after which time Hb synthesis was stopped by adding 5 ml of 5% trichloroacetic acid (TCA). The precipitated protein, mainly hemoglobin, was centrifuged and washed in 5 ml of diethlether. The dried protein pellet was counted for gamma activity in a well type Geiger-Mueller Counter (Electronics Corporation of India, Model LV-4755).

It is clear that under these experimental conditions, the counts obtained corresponds to amount of iron incorporated in protein, synthesized during 30 minutes of incubation period, which is mainly hemoglobin. Iron uptake and Hb synthesis was reported in cpm. Each protein pellet was counted thrice and average cpm were obtained. The counts were also corrected everyday for radioactive decay.

Effect of addition of iron : The effect of adding iron on Hb synthesis in anemic blood was also studied by above described technique (7). The test was run in duplicate simultaneously exactly as above with the only difference that in one of the tube 0.1 ml of appropriate form of iron was also added. The counts obtained in iron supplemented tube was compared with that of counts in tube without additional iron. The effect of following three forms of iron preparation was seen.

a) Ferrous iron as ferrous sulphate – 0.1 ml (=1μgFe) added.

b) Ferric iron as ferric chloride – 0.1 ml (=1μgFe) added.

c) Transferrin iron as normal plasma – 0.1 ml (=0.09μgFe) added

RESULTS AND DISCUSSION

The subjects of anemic group were suffering from severe anemia as evidenced by blood haemoglobin values (6.9 ± 0.7 g% Vs 13.0 ± 0.9 g% Hb) and other hematologic parameters. That iron deficiency was the

cause of this anenia was confirmed by values of plasma iron and percent saturation (SI, 32.6 ± 4.8 µg% Vs 106.4 ± 12.3 µg% and PS, 7.4 ± 1.6% Vs 33.1 ± 6.8% in anemic and control group respectively).

TABLE I : Radio iron incorporation into hemoglobin.

Group	*Iron incorporated*	
	cpm / ml blood	*cpm / million reticulocytes*
Control (26)	745±122	22.7±3.7
Anemic (29)	1222±125*	16.3±5.3*

*Values are statistically significant from control, P<0.001

The rate of hemoglobin synthesis in control and iron deficient groups is presented in Table 1. Since only reticulocytes synthesize Hb, the values are expressed as cpm/million reticulocytes, apart from cpm/ml blood. The rate of Hb synthesis in iron deficiency was apparently increased by 1½ times of control value ; however, this increase was due to increase in number of reticulocytes. Thus the rate of Hb synthesis was actually only two-third in iron deficient reticulocytes than control reticulocytes.

In order to determine whether iron was limiting factor in the impaired Hb synthesis observed in iron deficient reticulocytes, the experiments were repeated with supplemented iron (Table 2). Three forms of iron were used-ferrous, ferric and transferrin (normal plasma). The results show that the rate of Hb synthesis increased drastically by about 55% on addition of ferric iron and much more on transferrin iron. Surprisingly ferrous iron reduced Hb synthesis by about 30%. It may be due to the fact that if ferrous iron enters in the red cells, it rather inhibits transferrin iron uptake (4), as explained below.

$$Fe^{3+} \Leftrightarrow \overset{Fe^{3+}}{\text{Apotransferrin}} \overset{e^-}{\Uparrow} \Leftrightarrow Fe^{2+} + \text{Apotransferrin}$$

In above reaction, forward reaction will be favored by larger concentration of ferric iron while back ward reaction by larger concentration of ferrous iron according to the law of mass action.

The effect of ferric iron may be due to greater exchange of radioactive ferric iron with transferrin present in blood due to law of mass action or to greater percent saturation of transferrin, accomplished by added ferric iron. Increase in transferrin saturation is known to result in increased iron uptake and Hb synthesis (1). There was striking resemblance in the extent of

TABLE II : Effect of iron supplementation on hemoglobin synthesis by iron deficient reticulocytes.

Form of iron added	*Iron incorporated into Hb (cpm / million reticulocytes)*		*Percent change (%)*
	Without iron	*With iron*	
Ferrous (n=10)	17.9 ±5.0	12.5± 3.5*	–30%
Ferric (n=10)	17.9 ±5.0	27.8± 6.2*	+55%
Transferrin (n=10)	17.9±5.0	29.1± 5.8*	+62.5%

n denotes the number of cases.
*Statistically significant, P<0.001

increase in Hb synthesis by ferric or transferrin iron. It may also be noted that the resultant rate of Hb synthesis was significantly higher than even what was observed in the control group.

The transferrin (plasma) iron evoked Hb synthesis to the same extent as that of ferric iron, even in the one tenth molar concentration. On the other hand equimolar concentration of ferrous iron as that of ferric iron, reduced Hb synthesis considerably. All these observations suggest that unless iron present in plasma is oxidised to ferric state and accepted by transferrin it cannot be of any help in Hb synthesis.

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