# Erythropoietic Response to Hemolytic and Hemorrhagic Anemia[1] (36073)

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(Introduced by G. S. Hodgson)
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Control of erythropoiesis can be classified as a proportional system in which red blood cell (RBC) production is a decreasing function of tissue oxygen partial pressure (1). A decrease in hematocrit reduces the oxygen carrying capacity of the blood and determines an increased release of erythropoietin by the kidney (2). This hormone stimulates the differentiation and proliferation of red cell precursors (3). Erythropoietin concentration increases exponentially with decrease in hematocrit (4); and RBC production, over most of the observed range, is a linear decreasing function of hematocrit (1). The slope of the line relating RBC production to hematocrit seems to differ in hemolytic and hemorrhagic anemia, for RBC production at a given RBC concentration is greater in the former than in the latter condition (5). It has been suggested that the greater availability of iron to the marrow in hemolytic anemia could explain the difference (6). However other mechanisms could be involved, such as a stimulating effect of RBC breakdown products on erythropoiesis (7), perhaps as a consequence of an increase in stem cells (8, 9) and/or production of erythropoietin by the kidney (10).

The present study was designed to determine the effects of replacing iron lost in bleeding by iron dextran, on the relationship between RBC production and RBC concentration in hemorrhagic anemia compared to hemolytic anemia.

*Methods.* Male AxC rats, weighing between 180–200 g, were grouped in the following way: Group I, rats were bled by cardiac puncture, once a day for 3 successive days. This group was divided into subgroups of 5 rats according to the total amount of blood removed (ml): (a) 1.5; (b) 3.0; (c) 4.5; (d) no bleeding.

Group II, rats were bled in the same way and received daily ip injections of iron dextran (Inferon) in amount equal to the iron lost by bleeding (mg of Fe dextran): (a) 0.75; (b) 1.50; (c) 2.25; (d) no treatment. After cardiac puncture, plasma was reinjected to rats in groups I and II to restore the blood volume.

Group III, hemolysis was induced by daily injections of phenylhydrazine for 3 successive days, with the following dose schedules (five rats per subgroup) (mg/100 g body weight/day): (a) 0.6; (b) 1.2; (c) 2.4; (d) 4.8; (e) control, untreated.

Plasma iron turnover and $^{59}Fe$ incorporation into erythropoietic tissue were studied in each animal on day 6 by the method described previously (11). 0.2 $\mu$Ci of $^{59}Fe$ ($FeCl_3$) were injected iv; and 2 hr later, the animals were anesthetized and a blood sample was obtained by aortic puncture, with a heparinized syringe. The $^{59}Fe$ activity of plasma, RBC (thrice washed in saline), femurs, and spleen, was counted in a well-type scintillation counter (Nuclear Chicago).

From the $^{59}Fe$ distribution data, the fractional turnover rate of plasma iron $K$ ($hr^{-1}$), plasma iron turnover (PIT), and erythropoietic iron uptake (EIU), were calculated as described previously (11). The erythropoietic plasma iron clearance, was calculated as the ratio of EIU to plasma iron concentration. Plasma iron concentration was determined by the Ramsay method (12). The microhematocrit was measured in each animal.

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*Results.* The erythropoietic response as measured by erythropoietic iron uptake (EIU) is a decreasing linear function of hematocrit in all three groups. The slope of the line relating EIU to hematocrit (Fig. 1) is significantly ($p<.001$) greater in hemolytic $-0.670 \pm S_{yx}$ 1.04 than in hemorrhagic anemia, slope $-0.214 \pm S_{yx}$ 0.80. Iron dextran injection slightly increases the response of bled animals, slope $-0.342 \pm S_{yx}$ 0.35 ($p = .05$). Iron dextran-treated animals were also less anemic than the corresponding bled group (Table I). As expected, plasma iron concentration (Fig. 2, Table I) of the rats with hemolytic anemia was higher than that of bled rats, and, in general, above that of controls. Surprisingly, animals treated with iron dextran showed plasma iron concentration values lower than that of bled animals in the range of hematocrit obtained.

A significant linear relationship also exists between erythropoietic plasma iron clearance and hematocrit (Fig. 3). The points for the bled animals are below those of the other two groups. The slope of the line is greater for rats with hemolytic anemia than for bled rats

TABLE I. Comparison of Hematocrit and Plasma Iron Concentration in the Three Groups of Rats at Day 6.[a]

| Groups | Hematocrit (%) | Plasma iron conc ($\mu$g/ml) |
|---|---|---|
| I Hemorrhagic | | |
| Control | 39.0 ± 0.8 | 1.71 ± 0.26 |
| 1.5 ml | 34.4 ± 1.0 | 1.96 ± 0.17 |
| 3.0 ml | 31.2 ± 1.1 | 1.35 ± 0.10 |
| 4.5 ml | 28.0 ± 1.6 | 1.19 ± 0.31 |
| II Hemorrhagic + iron dextran | | |
| Control | 40.4 ± 0.3 | 1.64 ± 0.20 |
| 1.5 ml | 36.0 ± 0.7 | 1.34 ± 0.24 |
| 3.0 ml | 34.5 ± 0.5 | 1.81 ± 0.31 |
| 4.5 ml | 33.2 ± 0.5 | 1.14 ± 0.17 |
| III Hemolytic by phenylhydrazine | | |
| Control | 40.6 ± 0.5 | 1.95 ± 0.17 |
| 0.6 mg/100 g | 36.2 ± 0.7 | 2.32 ± 0.30 |
| 1.2 mg/100 g | 34.5 ± 0.8 | 1.96 ± 0.24 |
| 2.4 mg/100 g | 32.0 ± 0.8 | 2.47 ± 0.08 |
| 4.8 mg/100 g | 30.0 ± 0.7 | 1.87 ± 0.20 |

[a] Values recorded are mean ± SD.

$-0.327 \pm S_{yx}$ 0.37 and $-0.214 \pm S_{yx}$ 0.32, respectively. Bled rats treated with iron dextran have, for a given hematocrit, clearances indistinguishable from those of rats with hemolytic anemia, and the line relating clearance to hematocrit ($-0.300 \pm S_{yx}$ 0.70) is

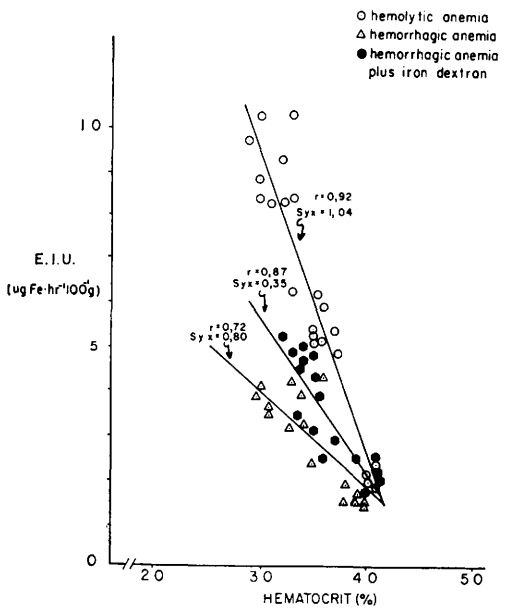


FIG. 1. Relation between erythropoietic iron uptake (EIU) and hematocrit: The lines represent regression equations. Each point represents one animal.

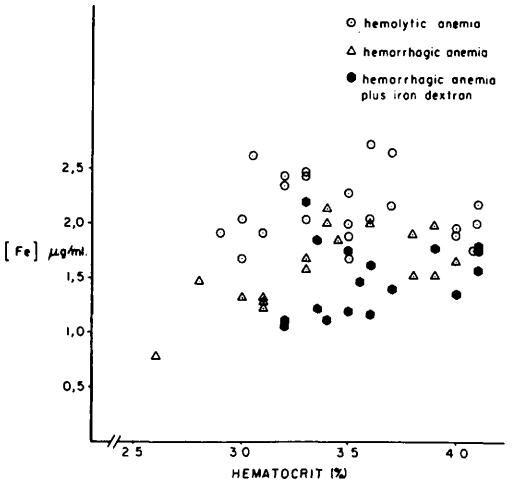


FIG. 2. Relation between plasma iron concentration ($\mu$g/ml) and hematocrit: Each point represents one animal.

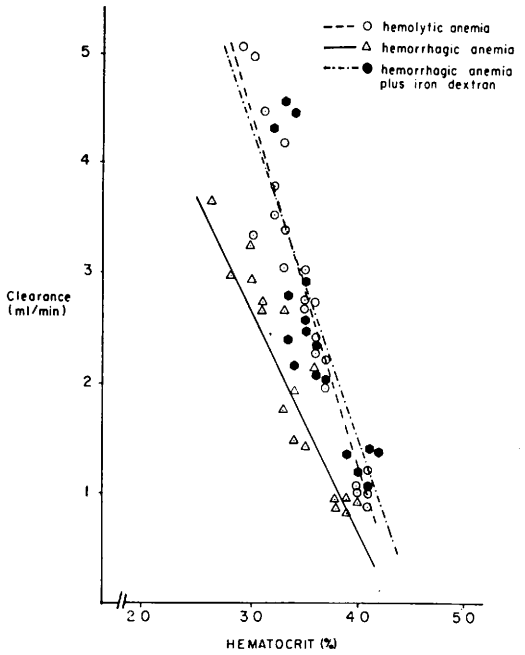


FIG. 3. Relation between erythropoietic iron clearance and hematocrit: Each point represents one animal. The lines represent regression equation.

the same as that for hemolytic anemia.

*Discussion.* The erythropoietic response to anemia as measured by iron uptake was a decreasing linear function of hematocrit in all three experimental situations. However the "gain" (slope of the line) of the system was the greatest in hemolytic anemia and smallest in hemorrhagic anemia. Injection of iron dextran to bled rats slightly increased the gain of the system and decreased the degree of anemia observed on day 6 after initiating treatment. The increased gain in rats treated with iron dextran was not accompanied by increased plasma iron concentrations as it was in hemolytic anemia.

Iron uptake by red cell precursors *in vitro* depends on the iron concentration, when it is below a limit value. Above this value iron uptake is maximum and independent of iron concentration (13). Similar results have been reported *in vivo* by Hodgson *et al.* (14) and more recently by Jacobs and Finch (15). In conditions when plasma iron is below the limit value, it has been postulated (14) that erythropoietic iron uptake is proportional to the product of the number of RBC precursors in marrow and plasma iron concentration:

$$\text{EIU} = \text{k} \cdot n\text{P} \cdot (\text{Fe}),$$

where $k$ = proportionality constant; $n$P = number of RBC precursors; and (Fe) = plasma iron concentration.

From this, it follows that the ratio of EIU to (Fe), or erythropoietic plasma iron clearance, is proportional to the number of RBC precursors present.

$$C = k \cdot n\text{P}$$

The fact that clearance is a linear function of hematocrit, even in rats with severe hemolytic anemia, suggests that in all groups iron concentration is below the limit value, for maximal uptake. Thus, the situation in phenylhydrazine-treated rats is different from that previously described for phenylhydrazine-treated rabbits (14) in which iron concentrations were over the limit value and, therefore, iron uptake was independent of iron concentration.

The differences in erythropoietic iron uptake between rats with hemolytic anemia and bled rats can be ascribed tentatively to both a difference in plasma iron concentration and erythropoietic plasma iron clearance, the latter presumably reflecting the total number of RBC precursors. In the case of iron dextran-treated bled rats, the difference can be ascribed only to a difference in plasma iron concentration since plasma clearance was the same as that of rats with hemolytic anemia. It appears then as if products of red blood cell breakdown and iron dextran increase the gain of the control system. This could in part be due to expansion of the stem cell pool, as was observed in phenylhydrazine-treated mice (8) and in mice receiving injections of heat-damaged RBC (9). It is conceivable that iron dextran, like saccharated iron oxide (16), might stimulate reticuloendothelial activity and an increase in the number of stem cells. This is amenable to experimental evaluation. Another possible mechanism for increased erythropoiesis in hemolytic anemia is an increase in kidney production of erythropoietin, which has been shown to occur after injection of hemolysates into rats but not in mice (10). It is possible that iron dextran has a similar effect.

*Summary.* Erythropietic iron uptake and erythropoietic plasma iron clearance were a linear decreasing function of hematocrit, both in hemolytic (phenylhydrazine) and hemorrhagic anemia. The gain of the system that is the slope of the line relating erythropoietic iron clearance to hematocrit was greater in hemolytic than in hemorrhagic anemia. Injection of iron dextran in bled rats, increased the gain of the system, without raising plasma iron concentration. Erythropoietic plasma iron clearance of bled rats treated with iron dextran was same as that of rats with hemolytic anemia.

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