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Research Article

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Control of Marrow Production by the Level of Iron Supply

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ABSTRACT The level of erythroid marrow production varies widely in different erythropoietic disorders. In part, this reflects the level of erythropoietin stimulation as determined by the severity of the anemia. However, iron supply plays an equally important role in the control of erythropoiesis. As demonstrated in normal individuals subjected to prolonged periods of phlebotomy-induced anemia, the erythroid marrow will increase production by as little as twice to as much as eight times normal, depending on the iron supply available from different iron pools. Whereas the iron delivered from normal reticuloendothelial stores or orally administered iron is sufficient for a marrow production response of only two to three times normal, the increased iron supply from nonviable red cells, hemolysis, or iron dextran infusions permits marrow production to rise acutely to levels of four to eight times normal.

INTRODUCTION

Although erythropoiesis has been extensively studied in a wide spectrum of disease states (1–7), there have been very few studies of the characteristics of the normal marrow response to varying degrees of anemia (8–15). The wide variation in the level of red cell production which occurs with different anemias has been considered to be a function of both the relative level of hematocrit depression and a variety of biochemical abnormalities and cofactor deficiencies which interfere with cell growth and maturation. The present studies have examined erythroid marrow production characteristics in normal men at varying levels of phlebotomy-induced anemia. A direct correlation between iron supply and marrow production was apparent. Depending on the iron supply available from different iron pools, the erythroid marrow may increase production by as little as twice normal to as much as eight times normal.

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METHODS

All studies were performed in the Clinical Research Center of King County Hospital, Seattle, Washington. Participants included two patients with hemochromatosis, normal volunteers, and patients with refractory gastrointestinal bleeding; all subjects were males between ages 23 and 48. Each individual was first evaluated to exclude renal impairment or significant illnesses which could have interfered with maximum marrow response to anemia. Throughout control and subsequent study periods, they were maintained on a high protein diet and folic acid supplements, 5 mg twice a day orally.

Initial studies (Table I) included: two measurements of plasma iron turnover, repeated serum iron (16) and total iron binding-capacity determinations (17), a ^{51}Cr-labeled red cell mass, reticulocyte counts, and a bone marrow examination with Prussian blue stain for iron stores. The subjects were then phlebotomized to a hematocrit of 32–37%. This level of anemia was maintained for 3–4 wk by daily phlebotomy adjusted to remove enough red cells to compensate for increased marrow production (18). Once a maximum marrow production plateau had been attained for at least 2 wk each subject was subsequently phlebotomized to a hematocrit of 25–30%. Again, the hematocrit level was kept constant for 3–5 wk until a second plateau in marrow production was obtained. During each period of phlebotomy, the marrow production response was determined from the level of daily phlebotomy, repeated plasma iron turnovers, and twice daily reticulocyte counts (18).

To study the effect on marrow production of varying iron supply, maximum marrow production was determined for several forms of iron supply in each individual (Table II). Iron was provided either as normal reticuloendothelial iron stores (i.e., those iron stores of the normal individual which are primarily involved in the cycle of red cell destruction and return of iron to the erythroid marrow), orally administered iron together with normal iron reticuloendothelial stores, orally administered iron alone after exhaustion of reticuloendothelial stores, the excessive stores of the hemochromatotic patient, iron dextran stores, or the iron available from infusions of nonviable red cells. Five individuals were studied at a time when they were relying on their own reticuloendothelial iron stores. Four individuals were given oral iron, 300 mg of ferrous gluconate every 2 hr while awake, for a total of 8–9 doses/day when their marrow reticuloendothelial iron stores had been depleted as judged from a Prussian blue stain of the marrow. The same dosage

TABLE I

Base line Data for the Six Normal Individuals and the Two Patients with Hemachromatosis

Subject	Hematocrit	Absolute reticulocyte count	Serum iron*	TIBC*	Plasma iron turnover		Utilization	Blood volumes	
						Index (xs normal)‡		Red cell mass	Total volume§
		%	μg/100 ml	μg/100 ml	mg/100 ml/day)		%	ml	ml
Normals									
1	47.5–45.0	0.8–1.1	113–124	383–390	0.78, 0.83	1.20, 1.28	92, 95.5	2190	5100
2	44.0–45.5	0.7–1.2	100–113	386–440	0.80, 0.77	1.23, 1.18	87, 103	1525	3780
3	42.0–47.0	1.3–1.6	76–95	405–434	0.81	1.25	80	2080	4941
4	38.0–40.0	0.6–0.9	52–70	441–480	0.72, 0.64	1.1, 1.0	95, 97	1750	4820
5	43.5–47.0	0.8–1.2	100–142	420–445	0.76, 0.68	1.17–1.04	84, 90	2200	5250
6	41.5–44.5	1.1–1.3	98–130	395–420	0.63, 0.82	0.97, 1.26	90, 88	1890	4710
Hemachromatosis									
1	45.5–48.0	1.2–1.8	380–395	410–423	0.88	1.35	53	2280	5350
2	39.0–42.0	0.9–1.2	262–290	310–335	0.68	1.05	62	1720	4550

* Range of values during the control period, including four to eight separate determinations.

‡ Calculated as per Giblett, Coleman, Pirzio-Biroli, Donohue, Motulsky, and Finch (1), employing 0.65 mg/100 ml of whole blood per day as a mean normal plasma iron turnover.

§ Calculated from the red cell mass.

schedule was used when oral iron was given in the presence of at least 2+ reticuloendothelial iron stores in two cases. Two patients with idiopathic hemochromatosis demonstrated 3+ or better marrow reticuloendothelial cell iron stores and large amounts of parenchymal cell iron on liver biopsy. Before study they had elevated iron values and demonstrated excessive urinary excretion of iron with injection of deferrioxamine. In two subjects iron supply from iron dextran was studied after intravenous infusion of 3 g of the material. The iron dextran[1] was diluted in 250 ml of 5% dextrose solution and infused over a 1 hr period without reaction. Marrow production response with iron provided as nonviable red cells was examined in two subjects by reinfusion of red cells removed each day after heating for 30 min at 50°C and by administration of their own red cells made nonviable by storage at room temperature for 4–5 days. These stored nonviable cells were checked for sterility 24 and 48 hr before reinfusion. 50 ml of stored red cells were infused every 6 hr for 10 days to provide approximately 200 mg of iron in the form of hemoglobin cleared into the reticuloendothelial cell system per day. Throughout this infusion period intravascular hemolysis was insufficient to deplete the subjects' haptoglobin. Since the marrow production response to iron dextran was characteristically different immediately after infusion while free iron dextran was present in circulation and when iron dextran had been completely sequestered in the reticuloendothelial cell, this difference in iron supply was further studied in patients with refractory, chronic gastrointestinal bleeding secondary either to hereditary telangiectasia or of unknown cause. Marrow production responses were determined at varying hematocrit levels in the period immediately after intravenous infusion 2–3 g of iron dextran and during the reticuloendothelial cell storage phase of the material. The level of marrow stimulation during these studies was kept constant by the same technique of graded daily phlebotomy for periods of 2–3 wk. Transferrin-bound iron levels were determined by the technique of antitransferrin antibody assay of total transferrin and unbound iron-binding capacity.[2] This method permitted the measurement of true transferrin-bound iron in the face of large concentrations of circulating iron dextran in the serum.

[1] Imferon, Lakeside Laboratories, Inc., Milwaukee, Wis.

RESULTS

Six normal individuals and two patients with hemochromatosis were studied in the normal state and after phlebotomy to hematocrit levels of 32–37% and subsequently 25–30%. Base line data for the eight individuals are shown in Table I. An example of the data obtained for one of the subjects during the periods of phlebotomy anemia is shown in Fig. 1. During the phlebotomy studies, iron was provided from several sources in order to study the relative availability of iron from different pools and the interaction of iron supply and marrow production. When possible, each subject was studied when iron was provided from two or more sources (Table II).

Marrow production at hematocrit level of 32–37% was studied on nine occasions, while the subjects relied on either their normal sources of iron supply, primarily reticuloendothelial cell iron stores in the normal individuals, oral iron, or, in the patients with hemochromatosis, a combination of reticuloendothelial plus increased parenchymal iron stores (Table III). In response to the sudden reduction of the hematocrit to 32–37%, marrow production rapidly increased over a 10 day period to reach a production plateau of 1.8–3.5 times normal. This plateau was considered to be the maximum level of marrow production for a hematocrit of

[2] Hillman, R. S. Direct iron exchange between iron dextran and transferrin. Data to be published.

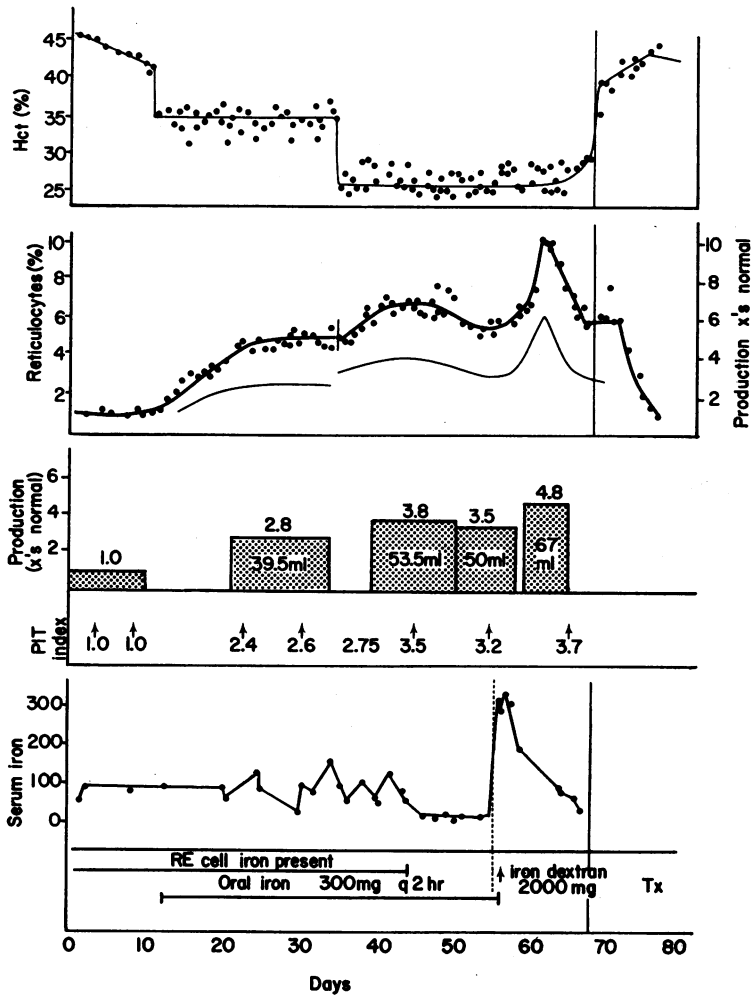

FIGURE 1 Serial measurements of marrow production from the level of daily phlebotomy (bar graph and solid line under the reticulocyte curve) and plasma iron turnovers (PIT index) were performed at two levels of phlebotomy anemia, hematocrits 35–37% and 25–27%. In this patient, iron was provided as reticuloendothelial cell iron stores plus oral iron, oral iron alone, and subsequently intravenous iron dextran.

approximately 35%, since production did not increase further when this level of anemia was maintained for 2–4 wk. The observed increases in red cell production were of a similar magnitude for all subjects despite the differences in iron supply. Although one of the patients with hemochromatosis did demonstrate a production level of 3.5 times normal, the presence of excessive iron stores and a high serum iron level did not necessarily promote a significantly higher level of marrow production than that seen in the individuals relying on either their reticuloendothelial iron stores or orally administered iron.

Maximum marrow production was subsequently determined for these same three patterns of iron supply after the hematocrit was reduced to 25–30%. In addition, subjects were studied when iron was provided from either a combination of reticuloendothelial iron stores plus oral iron, intravenous iron dextran, or nonviable red cells (Table IV). When iron supply was from normal reticuloendothelial stores or oral iron, maximum red cell production did not change significantly from that observed at a hematocrit of 32–37%. The two subjects who received no supplementation but relied on their normal iron supply mechanisms reached a maximum production level of 2.8 and 3.2 times normal. Those subjects with no observable iron stores on Prussian blue stain of the marrow who were maintained on oral iron, 300 mg of ferrous gluconate q 2 hr, demonstrated a

TABLE II

Sources of Iron Supply

Subjects...	1	2	3	4	5	6	7*	8*
Reticuloendothelial iron stores, alone	x	x	x		x	x		
Reticuloendothelial iron stores plus oral iron	x	x			x	x		
Oral iron, alone		x	x	x		x		
Reticuloendothelial iron plus parenchymal iron stores							x	x
Iron dextran	x	x						
Nonviable red cells			x	x				

* Patients with hemachromatosis.

slight increase in production to levels of 2.9–4.0 times normal. In both situations, frequent serum irons were depressed to levels of 20–105 μg/100 ml. In contrast, when iron was provided from a combination of sources or as either iron dextran or nonviable red cells, production increased to levels above four times normal. When the normal individuals with 2+ or better reticuloendothelial iron stores were given oral iron supplements, marrow production increased to 4.1 and 4.8 times normal. In one of the patients with hemochromatosis (reticuloendothelial iron and increased parenchymal iron stores), maximum red cell production reached a level of 5.2 times normal. Again, the serum iron level was depressed below 100 μg/100 ml despite the additional sources of iron supply.

TABLE III

Marrow Production Response to a Hematocrit of 32–37%

Iron supply	Subjects	Serum iron* Mean	Serum iron* Range of values	Marrow production‡ (xs normal)
		ug/100 ml		
Iron supply of the normal individual, primarily reticuloendothelial iron stores	5	112	85–150	1.8–2.8
Oral iron, alone (after depletion of marrow reticuloendothelial iron stores)	2	123	105–160	2.8, 3.0
Reticuloendothelial plus increased parenchymal iron stores (i.e., the iron stores of the patients with hemochromatosis)	2	245	220–280	2.7, 3.5

* Mean and range of values for 6–10 serum iron determinations for each subject, obtained during the period of production plateau.

‡ Marrow production as measured by phlebotomy balance and at least two determinations of the plasma iron turnover during the period of production plateau.

TABLE IV

Marrow Production Response to a Hematocrit of 25–30%

Iron supply	Subjects	Serum iron* Mean	Serum iron* Range of values	Marrow production‡ (xs normal)
		μg/100 ml		
Iron supply of the normal individual, primarily reticuloendothelial iron stores	2	44	25–70	2.8, 3.2
Oral iron, alone (after depletion of marrow reticuloendothelial iron stores)	4	65	20–105	2.9–4.0
Reticuloendothelial iron stores plus oral iron	2	104	60–120	4.1, 4.8
Reticuloendothelial plus increased parenchymal iron stores (hemochromatosis)	1	84	70–100	5.2
Iron dextran, immediately after infusion	3	315	270–450	4.5–5.5
Iron dextran, reticuloendothelial stores	3	78	45–125	2.5–3.5
Nonviable red cells	2	235	200–275	6.6, 7.8

* Mean and range of values for three to eight serum iron determinations for each subject.

‡ Marrow production as measured by a combination of phlebotomy balance, plasma iron turnover, and reticulocyte production indices.

On five occasions, iron was supplied either as intravenous iron dextran or by repeated infusion of nonviable red cells. This was followed by a rise in the serum iron to levels greater than 200 μg/100 ml and an increase in marrow production to 4.5–7.8 times normal within the next 10 days. Since the iron supply from these sources was not maintained for longer than 10 days, a maximum production plateau was not measured. The production levels listed in Table IV for these two types of iron supply were obtained at a time when production was still increasing and represent the peak level of production observed.

As shown in Table IV and Fig. 2, the serum iron elevation and increased marrow production after the infusion of iron dextran was a transient phenomenon. After 7–10 days, when the iron dextran had been sequestered in the reticuloendothelial cell, the serum iron returned to values of less than 120 μg/100 ml and marrow production fell to a level of 2.5–3.5 times normal. This correlation of the serum iron level with the marrow production response is evident for the entire group of patients phlebotomized to a hematocrit of 25–30% (Fig. 3). In those

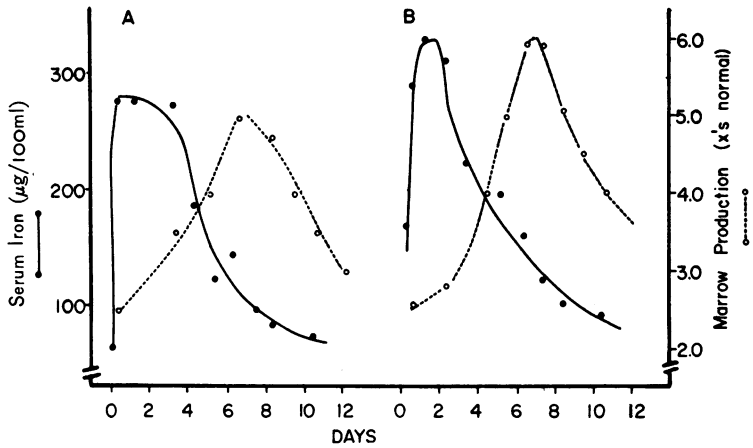

FIGURE 2 Serum iron and marrow production indices in two subjects after infusion of 2000 mg of iron dextran. The serum iron was measured by the antitransferrin antibody technique.[2] Marrow production was measured by both the phlebotomy production measurement and reticulocyte production index.

subjects in whom the mean serum iron was below 70 μg/100 ml, maximum red cell production remained between 2.5 and 3.5 times normal. With serum iron values between 70 and 150 μg/100 ml, production was able to increase to four to five times normal. Only when iron supply was sufficient to transiently increase the serum iron to values greater than 200 μg/100 ml did marrow production increase to levels of 4.5–7.8 times normal.

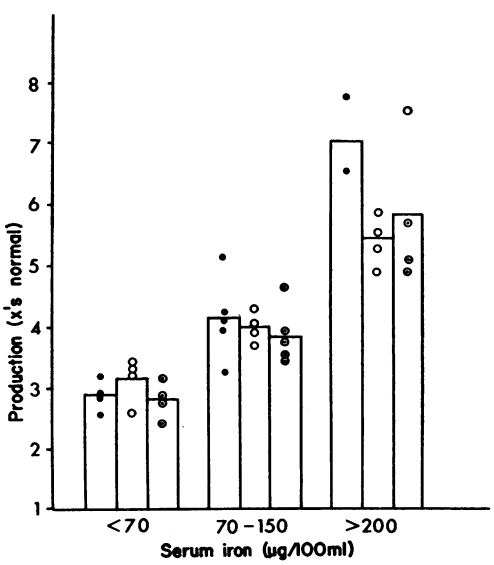

FIGURE 3 Marrow production at a hematocrit level of 25–27% was directly related to the serum iron level. Production was determined by three techniques: plasma iron turnover (closed circles), phlebotomy production measurement (open circles), and reticulocyte production index (circled dots).

DISCUSSION

Despite extensive measurements of erythroid marrow production levels in pathological anemic states, very few measurements of normal marrow production characteristics have been performed. Coleman, Stevens, Dodge, and Finch (13) and Finch, Haskins, and Finch (14) reported marrow production rates of 1.5–2 times normal in individuals phlebotomized 500–1000 ml of whole blood at weekly intervals. In these studies, the subjects' hematocrits ranged between 34 and 39%. They also demonstrated that the onset of iron deficiency in these individuals was associated with a fall in marrow production to normal or less than normal marrow production levels. Crosby (12) demonstrated a maximum marrow production response of three to six times normal in a single individual undergoing rapid phlebotomy for treatment of hemochromatosis. In hemolytic anemia patients he was able to show a similar level of maximum marrow production (7).

In the present studies, the normal erythroid marrow response to specific levels of anemia has been examined further with special attention directed to the influence of iron supply on marrow production. At a hematocrit level of 32–37%, red cell production increased to 1.8–3.5 times normal and remained at this level in all subjects regardless of differences in available iron pools. Although the two patients with the excessive iron stores of hemochromatosis maintained their serum irons between 220 and 280 μg/100 ml as compared to 85–160 μg/100 ml for the normal subjects, they did not demonstrate significantly greater red cell production. However, when the hematocrit was lowered to 25–30%, the erythroid marrow response did appear to reflect dif-

ferences in iron supply. Individuals relying primarily upon reticuloendothelial iron stores or oral iron were unable to increase their marrow poduction above four times normal. Only when iron was provided from a combination of sources or as either intravenous iron dextran or nonviable red cells did marrow production increase to levels of four to eight times normal.

From these results, it is apparent that the rate of iron delivery to the marrow is a major factor in the regulation of marrow proliferation. When iron supply is restricted and the serum iron value falls below 70 μg/100 ml an obvious limitation of marrow production is observed. If iron delivery is improved because of the existence of two or more iron supply pools or the presence of significantly increased breakdown of red cells, marrow production can increase to levels of four to eight times normal. Although the highest levels of production in the present studies were accompanied by an elevation in the serum iron to levels well above 200 μg/100 ml, this may not be essential. As shown in studies of hemolytic anemias by Crosby and Akeroyd (7), marrow production can increase to five to six times normal at a time when the serum iron is between 100 and 200 μg/100 ml. Therefore, it is possible that the high serum iron values of the present studies represent a transient imbalance between iron supply and marrow proliferation. If marrow proliferation had been permitted to attain a maximum plateau, the rate of iron uptake and iron delivery to the marrow may well have reached a balance so as to return the serum iron to levels below 200 μg/100 ml. In this case the measurement of maximum marrow production may well have been too low. Since it was impossible to maintain the high values of serum iron for longer than 10 days, a maximum level of production was not obtained. Production was still increasing when the study was terminated.

For this reason, it is more appropriate to correlate the level of marrow production with the characteristics of the available iron supply. Thus the normal 60–70 kg individual who derives the majority of the iron required for increased erythropoiesis from his reticuloendothelial iron stores will deliver a maximum of 40–60 mg of iron per day to marrow precursors when his hematocrit is 25–30%. Why this limitation exists is unclear. It may either reflect a limitation of membrane transport and attachment of iron to transferrin which is innate to the reticuloendothelial cell or be the result of the time required for dissolution of the hemosiderin storage form. Oral iron alone, in dosages of 300 mg of ferrous gluconate every 2 hr while awake, will provide a maximum of 60–80 mg of iron per day. When two iron sources are present such as reticuloendothelial iron stores plus oral iron or reticuloendothelial iron stores plus parenchymal iron the amount of iron delivered to the marrow may reach 80–100 mg/day. In contrast, nonviable red cells or infusions of iron dextran provide significantly more iron for transport to the marrow; production can rise to at least 4.5–7.8 times normal for a delivery rate of 80–160 mg of iron per day. The iron supply from hemolyzed red cells was especially efficient in this regard. This fact is of considerable interest since the reticuloendothelial cell has the responsibility of recovering the iron from the nonviable red cell and returning it to transferrin for delivery to the marrow. It would imply a more rapid transport of iron across the cell membrane with hemolysis than was seen with mobilization of hemosiderin reserves, which by itself or perhaps in association with an erythropoietin-like effect of the porphyrin and amino acid breakdown products of hemolysis results in a higher level of marrow production.

These findings extend the understanding of the role iron plays in controlling marrow production. Obvious iron deficiency in which iron stores are exhausted and serum irons fall below 40 μg/100 ml is not necessary for restriction of marrow production. As the level of marrow production increases, iron supply must keep pace, otherwise maximum production capabilities will not be realized. For any disease state, this permissive level of iron supply will vary depending on the number and characteristics of available iron pools. Whereas with a hemorrhagic anemic, normal reticuloendothelial stores permit only three to four normal production, a hemolytic process will be able to attain six to seven times normal marrow activity because of the rapid delivery of iron from the destroyed red cells. This phenomenon may well explain many of the production variations in pathological states in which there are varying admixtures of hemolysis and reticuloendothelial iron storage.

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