Studies on Iron Absorption. I. The Relationships Between the Rate of Erythropoiesis, Hypoxia and Iron Absorption

By GERALD A. MENDEL

Since the demonstration by McCance and Widdowson of the marked limitations of human iron excretion, it has been recognized that under normal conditions total body iron in man is maintained at a relatively constant level by some mechanism which regulates the amount of iron absorbed from the gastrointestinal tract.

In spite of intensive investigation, the mechanisms that control iron absorption are incompletely defined. The once widely accepted concept, advanced by Hahn and Granick, of a “mucosal block” as the prime regulatory mechanism whereby saturation of the mucosal cell with ferritin impedes further iron absorption no longer appears tenable in view of more recent studies made possible by technological advances. Brown has shown that the “block” to absorption induced by prior iron feeding is not complete and has suggested that it is relatively unimportant as a regulatory mechanism when physiologic quantities of iron are involved. Heilmeyer has demonstrated a rising ferritin content in the liver following iron ingestion at a time when mucosal ferritin content was maximal.

Other factors which are regarded as important in the regulation of iron absorption include the rate of erythropoiesis, bowel hypoxia, the content of total-body iron, the activity of iron-containing enzymes and the state of the plasma iron-transferrin system.

The rate of erythropoiesis has come to be considered one of the more important internal regulators of iron absorption in man under “physiologic” conditions. Numerous conditions are known, both in human subjects and in experimental animals, in which an acceleration of red cell production is paralleled by an increase in the gastrointestinal absorption of iron. These conditions include anoxic hypoxia, cobalt administration, hemolytic anemias of varied etiology and the anemia induced by blood loss. In contradistinction, iron absorption is increased in patients with hemochromatosis without measurable acceleration of the rate of erythropoiesis.

It has been demonstrated repeatedly that hypoxic anoxia enhances iron absorption in in vivo experiments. In vitro studies using everted sacs of rat duodenum, however, anaerobic conditions were found to impede iron transport across the mucosa. It has frequently been tacitly assumed that the
increased absorption of iron associated with hypoxia is secondary to increased red cell production. The role and importance of anoxia, hypoxic and anemic, in iron absorption remain uncertain.

The present studies were undertaken to explore further the relationships between the rate of red cell production, hypoxia and the gastrointestinal absorption of iron.

MATERIALS AND METHODS

CF No. 1 virgin female mice 9–12 weeks old, maintained on a Rockland Mouse Pellet diet, were used in all experiments except as indicated in table 5 where mice 20–30 weeks old were used. Water containing approximately 12 μg. of iron per ml. was given for 3 weeks prior to the beginning of an experiment.

Splenectomy was performed through a small left flank incision under intraperitoneal sodium pentobarbital anesthesia.

Mice treated with radiostrontium were given 2 or 4 μc. of Sr⁸⁹ Cl₃ per Gm. of body weight intraperitoneally on the day following splenectomy and eleven days prior to the feeding of Fe⁵⁹ SO₄. As has been shown by Jacobson et al. the excretion of Sr⁸⁹ was negligible after this 11-day period.

Mice were hypertransfused by intraperitoneal injection of 0.5 cc. of washing homologous red blood cells twice daily for 2 days beginning the day after Sr⁸⁹ administration.

Mice treated with erythropoietin were given 6 units of erythropoietin subcutaneously daily for 3 days beginning 6 days after red cell transfusion. The 6-day interval was chosen to coincide with the period of maximum suppression of erythropoiesis induced by hypertransfusion. The unit of activity is as described by Goldwasser and White. The preparation of the erythropoietin has been described elsewhere.

Hypoxic anoxia was induced in animals by placing them in a chamber in which a gas mixture containing 10 per cent O₂ was maintained. The animals were placed in this chamber for 24 hours before and after the administration of Fe⁵⁹ intragastrically or intravenously.

Iron absorption was measured by total body counting in a Geiger-Mueller well counter according to the method of Krantz et al. Fasting mice were force-fed 0.6 cc. of a solution of .01 N HCl containing 1.5 μc. Fe⁵⁹ SO₄ and 36 μg. of FeSO₄·7H₂O.

In mice treated with strontium-89, percentage absorption of Fe⁵⁹ was calculated from the formula:

\[
\% \text{ Fe}^{59} \text{ absorbed} = \frac{C - k_1 A}{k_2 (B - A)}
\]

Where: 
A = cpm due to Sr⁸⁹ immediately prior to Fe⁵⁹ feeding
B = cpm immediately after Fe⁵⁹ feeding
C = cpm 3 days after Fe⁵⁹ feeding
\( k_1 \) = decay factor for Sr⁸⁹
\( k_2 \) = decay factor for Fe⁵⁹

Because of variations in mean iron absorption values of normal mice in different experiments, no attempt has been made to make comparisons between absolute values obtained in these various experiments. No correction was made for contamination of the Sr⁸⁹ with Sr⁹⁰. Calculation indicated that this contamination could introduce a maximum error of +0.4 per cent in animals receiving radiostrontium.

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*Nucleonic Corporation of America Model WC-3.
Table 1.—The Effect of Erythropoietin on Iron Absorption in Hypertransfused Mice

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>% Fe&lt;sup&gt;59&lt;/sup&gt; ABSORBED FROM G-I SHAFT</th>
<th>At time of Fe&lt;sup&gt;59&lt;/sup&gt; feeding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean reticulocyte count</td>
<td>Mean hematocrit</td>
</tr>
<tr>
<td>NORMAL CONTROLS (9)*</td>
<td>28.8 ± 5.03**</td>
<td>5.55%</td>
</tr>
<tr>
<td>HYPERTRANSFUSION (8)</td>
<td>6.3 ± 3.85</td>
<td>0.03%</td>
</tr>
<tr>
<td>HYPERTRANSFUSION + ERYTHROPOIETIN (9)</td>
<td>12.1 ± 1.46</td>
<td>2.20%</td>
</tr>
</tbody>
</table>

* Number of mice in each group  
** Standard error of mean

Iron incorporation into circulating red blood cells was determined by injecting 1 μc. of Fe<sup>59</sup> citrate into the tail vein of a mouse and measuring the radioactivity in 0.2 cc. of blood approximately 24 hours later. Calculations of percentage incorporation were based on assumed blood volume of 6 per cent. Changes in blood volume produced by anemia and hypoxia were considered insignificant.

It should be noted that in these experiments iron absorption was measured over a 3-day period to allow time for elimination of unabsorbed radioiron from the gut. The incorporation of intravenously-administered iron was measured during the 24-hour period coinciding with the time of maximum iron absorption.

Reticulocyte counts were done on tail vein blood by the direct smear method using brilliant cresyl blue and counting 1000 red cells. Considerable variation in normal count was found to exist in groups of mice of identical age received at various times during the year. Variation within a group was not large.

Hematocrits were determined on tail vein blood collected in heparinized capillary tubes and spun in a microhematocrit centrifuge.

Histologic studies were performed on mice killed by cervical fracture. Tissues were fixed in Zenker-formol, embedded in nitrocellulose, cut at 6–8 μ and stained with hematoxylin-cosin-azure and or Prussian blue. Whole mounts of femur and specimens of small intestine, liver and spleen were obtained.

Results

The Effect of Erythropoietin on Iron Absorption in Hypertransfused Mice

To suppress red cell production, groups of mice were hypertransfused. By the sixth day after hyper-transfusion, erythropoiesis was markedly reduced as evidenced by a fall in the reticulocyte count to near zero and by the disappearance of proerythroblasts and normoblasts from the bone marrow and spleen. When such hypertransfused mice are given three doses of erythropoietin at 24-hour intervals, a wave of erythropoiesis sweeps through the marrow with a maximal rise in reticulocytes during the third day and a return in the reticulocyte count to zero by the seventh day. Iron absorption was measured during this period of accelerated erythropoiesis in the erythropoietin stimulated mice.

As indicated in table 1, mean iron absorption in the normal controls was 28.8 per cent and the average reticulocyte count was 5.55 per cent. Iron absorption in the hypertransfused group was reduced to a mean of 6.5 per cent and red cell production was markedly supressed as indicated by the mean reticulocyte count.

*It is recognized that the iron content of the injected cells may play some role in the reduction of absorption in both hypertransfused groups.
reticulocyte count of 0.03 per cent. In the erythropoietin-treated hypertransfused mice, the absorption of iron was increased from the mean of 6.3 per cent to 12.1 per cent, paralleling the increase in red cell production indicated by the rise in the mean reticulocyte count from 0.03 per cent to 2.20 per cent.

The Effect of Erythropoietin on Iron Absorption in Hypertransfused Mice with Erythropoietic Suppression Produced by Splenectomy and Sr59 Administration

To determine whether the action of erythropoietin in enhancing iron absorption was directly on the gastrointestinal mucosa or was dependent on increased red cell production, groups of mice were injected with 2 μc./Gm. of Sr59 to impair the capacity of the bone marrow to respond to erythropoietin. Figure 1 illustrates the almost complete replacement of normal marrow by fibrous tissue in a Sr59 treated animal. Jacobson17 has shown that mice given this dose of Sr59 rapidly develop a marked increase in erythropoiesis in the spleen. For this reason splenectomy was performed on the day prior to strontium-89 administration.

*It is unlikely that splenectomy followed by this dose of Sr59 results in complete suppression of erythropoiesis. The anemia which develops in mice so treated does not become fully expressed until 4 weeks following radiostrontium administration and is only moderate in degree.
Table 2.—The Effect of Erythropoietin on Iron Absorption in Splenectomized-Sr$^{89}$ Treated Hypertransfused Mice

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>% Fe$^{59}$ ABSORBED FROM G-I TRACT</th>
<th>At time of Fe$^{59}$ feeding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean reticulocyte count</td>
<td>Mean hematocrit</td>
</tr>
<tr>
<td>NORMAL CONTROLS</td>
<td>(9)**</td>
<td>28.8 ± 5.03***</td>
</tr>
<tr>
<td>SPLENECTOMY + Sr$^{89}$</td>
<td>(8)</td>
<td>18.1 ± 2.47</td>
</tr>
<tr>
<td>SPLENECTOMY + Sr$^{89}$ + HYPERTRANSFUSION</td>
<td>(7)</td>
<td>7.2 ± 1.32</td>
</tr>
<tr>
<td>SPLENECTOMY + Sr$^{89}$ + HYPERTRANSFUSION + ERYTHROPOIETIN</td>
<td>(8)</td>
<td>5.2 ± 0.91</td>
</tr>
</tbody>
</table>

* Sr$^{89}$ 2 μg/gm
** Number of mice in each group
*** Standard error of mean

As shown in table 2, the mean iron absorption value in the splenectomized-Sr$^{89}$-treated group was 18.1 per cent and the mean reticulocyte count in this group was 1.27 per cent. In the splenectomized-Sr$^{89}$ hypertransfused group, mean absorption was reduced to 7.2 per cent and the mean reticulocyte count was .01 per cent. In the erythropoietin-treated group, mean absorption was 5.2 per cent and reticulocytes were 0.25 per cent. Thus, in the erythropoietin-stimulated, hypertransfused group pretreated by splenectomy and Sr$^{89}$, there was no increase in iron absorption or in reticulocyte count comparable to that demonstrated in table 1.

The Effect of Erythropoietin on Iron Absorption in Hypertransfused Mice with Intact Spleens Treated with Sr$^{89}$

Figure 2 illustrates the marked increase in blood formation in the spleen that occurs in an animal treated with 2 μc./Gm. of Sr$^{89}$ in which the spleen was left intact. As indicated in table 3, no significant difference in iron absorption was found between animals with intact spleens treated with 2 μc./Gm. of Sr$^{89}$ and the normal controls. However, the enhancing effect of erythropoietin on iron absorption previously observed in hypertransfused mice with intact spleens (see table 1) was not observed in hypertransfused mice with intact spleens treated with Sr$^{89}$. The mean absorption value with the Sr$^{89}$ treated hypertransfused group with intact spleens was 3.7 per cent and the mean absorption value with the Sr$^{89}$ treated hypertransfused group with intact spleens treated with erythropoietin was 4.7 per cent.

The Effect of Erythropoietin on Iron Absorption in Hypertransfused Mice Pretreated by Splenectomy Alone

As demonstrated in table 4, splenectomy alone had no significant effect on iron absorption. Hypertransfused mice previously splenectomized showed a reduction in iron absorption from a mean value of 24.8 per cent to 3.4 per cent. Splenectomized and hypertransfused mice given erythropoietin exhibited only a slight increase in absorption from a mean value of 3.4 per cent.
to 4.6 per cent. While the difference shown is not statistically significant, it is typical of the results obtained in several experiments.

The Effect of Hypoxia on Iron Absorption and Erythropoiesis in Normal and Splenectomized-Sr°° Treated Mice

To determine whether the reported effect of hypoxia in increasing iron absorption is dependent on acceleration of the rate of erythropoiesis, the effect of decreased oxygen tension on iron absorption and erythropoiesis in normal and splenectomized mice treated with 4 μc./Gm. Sr°° was studied.

Table 5 shows that normal mice made hypoxic exhibited a marked increase in iron absorption from a mean value of 15.5 per cent to 33.1 per cent as well as an increase in red cell production from a mean of 38.8 per cent* to 56.5 per cent as measured by the incorporation of intravenously administered Fe°° into newly released circulating red cells. The splenectomized-Sr°° treated mice that were made hypoxic likewise showed a marked increase in iron absorption from a mean value of 11.6 per cent to 30.3 per cent, but showed no change in the rate of red cell production, which remained markedly reduced at a mean value of 4.0 per cent. Comparable results were obtained in mice treated with 2 μc./Gm. of Sr°° and in which the degree of anemia was much less.

*In other experiments, 24-hour incorporation of I.V. Fe°° was as low as 15 per cent in normal animals of the same age.
Table 3.—The Effect of Erythropoietin on Iron Absorption in Hypertransfused Mice with Intact Spleens Treated with Sr\(^{89}\)

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>% Fe(^{59}) ABORBED FROM G-I TRACT</th>
<th>At time of Fe(^{59}) feeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>NORMAL CONTROLS (10)**</td>
<td>15.3 ± 2.10***</td>
<td>2.20%</td>
</tr>
<tr>
<td>Sr(^{89})</td>
<td>18.0 ± 2.96</td>
<td>2.97%</td>
</tr>
<tr>
<td>Sr(^{89}) + HYPERTRANSFUSION</td>
<td>3.7 ± 0.60</td>
<td>0.00%</td>
</tr>
<tr>
<td>Sr(^{89}) + HYPERTRANSFUSION +</td>
<td>4.7 ± 1.10</td>
<td>2.01%</td>
</tr>
<tr>
<td>ERYTHROPOIETIN</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* = Sr\(^{89}\) 2 μg/gm
** Number of mice in each group
*** Standard error of mean

Table 4.—The Effect of Erythropoietin on Iron Absorption in Hypertransfused Mice Treated with Splenectomy

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>% Fe(^{59}) ABORBED FROM G-I TRACT</th>
<th>At time of Fe(^{59}) feeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>NORMAL CONTROLS (10)*</td>
<td>24.8 ± 2.22**</td>
<td>1.63%</td>
</tr>
<tr>
<td>SPLENECTOMY</td>
<td>23.6 ± 1.43</td>
<td>2.60%</td>
</tr>
<tr>
<td>SPLENECTOMY + HYPERTRANSFUSION</td>
<td>3.6 ± 0.85</td>
<td>0.00%</td>
</tr>
<tr>
<td>SPLENECTOMY + HYPERTRANSFUSION +</td>
<td>4.6 ± 0.93</td>
<td>1.45%</td>
</tr>
<tr>
<td>ERYTHROPOIETIN</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Number of mice in each group
** Standard error of mean

DISCUSSION

The present demonstration that iron absorption is enhanced by the hormone erythropoietin is in accord with the observations of Moore\(^6\) and Bothwell\(^7\) and their co-workers on the relationship that exists between the rate of erythropoiesis and iron absorption. Our data indicate that the mechanism by which erythropoietin augments iron absorption lies not in a direct action on the gastrointestinal mucosa, but is dependent on acceleration of erythropoiesis. Under the experimental conditions reported, augmentation of iron absorption by erythropoietin did not occur when the ability to increase red cell production was impaired by splenectomy and radiostrontium. Radiostrontium was chosen because of its rapid localization in bone and in an attempt to avoid radiation injury to the gastrointestinal mucosa. That this attempt was successful is suggested by the finding that iron absorption was normal in animals treated with Sr\(^{89}\) in which the spleen (now the major organ of blood formation) was left intact. The rate of erythropoiesis, as measured by the incorporation of intravenously administered radiciron into newly released circulating red cells did not vary significantly from normal in this preparation.\(^2\) These two findings make it unlikely that radiostrontium itself, in the doses used, affects absorption or that a systemic inhibitor to erythropoietin exists in the radiostrontium-treated animals.

The diminution of the enhancing effect of erythropoietin on iron absorption
Table 5.—The Effect of Hypoxia on Iron Absorption and Erythropoiesis in Normal and Splenectomized-Sr\textsuperscript{-89} Treated Mice

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>( % \text{Fe}^{59} \text{ABSORBED} )</th>
<th>( % \text{IV-INJECTED} \text{Fe}^{59} \text{CYCLED} \text{RED} \text{CELLS} )</th>
<th>At time of ( \text{Fe}^{59} \text{TRACING} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>NORMAL CONTROL</td>
<td>15.5 ± 1.90** (8)</td>
<td>38.8 ± 2.57 (8)</td>
<td>4.095 ± 0.03 (6)</td>
</tr>
<tr>
<td>NORMAL HYPOXIA</td>
<td>56.5 ± 4.85 (8)</td>
<td>4.215 ± 0.03 (6)</td>
<td>0.295 ± 0.03 (6)</td>
</tr>
<tr>
<td>SPLENECTOMY + Sr\textsuperscript{-89}</td>
<td>12.6 ± 1.30 (10)</td>
<td>4.1 ± 0.36 (6)</td>
<td>0.195 ± 0.03 (6)</td>
</tr>
<tr>
<td>SPLENECTOMY + Sr\textsuperscript{-89} + HYPOXIA</td>
<td>30.1 ± 4.18 (8)</td>
<td>4.0 ± 0.00 (6)</td>
<td>0.145 ± 0.03 (6)</td>
</tr>
</tbody>
</table>

* \( \% \text{Fe}^{59} \text{g/mg} \)
** Standard error of mean
*** Number of animals in each group

in radiostrotium-treated animals with intact spleens and in animals subjected to splenectomy alone remains unexplained. One of several possible explanations is that this is a reflection of the reduction in the stem cell pool entailed by these procedures, and one may speculate that cellular division at an immature stage in some manner influences the mucosa. Of some interest in this connection are (1) the suggestion by Mazur\textsuperscript{22} and his co-workers that the incorporation of plasma iron into ferritin is linked to oxidative metabolism, nucleoprotein, and adenosine triphosphate synthesis, and (2) their demonstration of increased incorporation of plasma iron into ferritin in animals undergoing liver regeneration as well as in animals treated with thyroxine.

The demonstration that iron absorption in hypoxic animals is greatly enhanced in the presence of profound suppression of erythropoiesis and without experimental manipulation likely to alter total body iron content emphasizes the existence and importance of factors influencing absorption that are independent of accelerated erythropoiesis and total body iron content \textit{per se}. These data clearly demonstrate for the first time that increased absorption due to hypoxia can occur independently of acceleration of erythropoiesis. The effects of chronic iron deficiency, acute blood loss, cobalt administration, and pyridoxine deficiency in augmenting iron absorption, and the role of the concomitant acceleration of erythropoiesis warrant further exploration in the light of these findings.

The important question whether the augmenting effect of hypoxia on iron absorption is due to a direct effect on the mucosa or is a reflection of the many changes induced by hypoxia elsewhere in the organism is unanswered. Pertinent to this question are the reported effects of hypoxia in producing a decrease in serum iron concentration and elevation of the serum transferrin concentration,\textsuperscript{23} the production of changes in iron-containing enzyme systems and other metabolites\textsuperscript{24,25} as well as the relationship of oxidative metabolism to the internal movements of iron.\textsuperscript{26}

There can be little doubt that multiple factors are operative in determining how much iron will be absorbed at any point in time. We believe that the splenectomized, strontium-89 treated animal will be of further use in the more precise definition of these factors.
STUDIES ON IRON ABSORPTION I

SUMMARY

(1) Evidence is presented that the augmentation of iron absorption from the gastro-intestinal tract produced by hypoxia can occur independently of acceleration of erythropoiesis.

(2) It is demonstrated that the hormone erythropoietin is capable of enhancing iron absorption; and that this property of erythropoietin is indirect, being dependent on acceleration of erythropoiesis.

(3) An experimental model for the study of those factors that affect iron absorption and are independent of increased red cell production is presented.

SUMMARIO IN INTERLINGUA

1. Es presentate datos que indica que le augmentation del absorption de ferro ab le vias gastro-intestinal que es causate per hypoxia pote occurrer independentemente de un acceleration del erythropoiese.

2. Es demonstrate que le hormon erythropoietina es capace de promover le absorption de ferro e que iste proprietate de erythropoietina es indirecte in tanto que illo depende de un acceleration del erythropoiese.

3. Es presentate un modello experimental pro le studio de ille factores que affice le absorption de ferro e que es independente de un augmento in le production de erythrocytos.

ACKNOWLEDGMENT

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REFERENCES


Gerald A. Mendel, M.D., Instructor, Department of Medicine, University of Chicago, and Argonne Cancer Research Hospital, Chicago, Ill.
Studies on Iron Absorption. I. The Relationships Between the Rate of Erythropoiesis, Hypoxia and Iron Absorption

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