Independence of Iron Absorption From the Rate of Erythropoiesis

By C. Peschle, G. P. Jori, G. Marone, and M. Condorelli

The regulation of iron absorption is only partially understood. A cause and effect relationship between the erythropoietic and the absorption rates has been postulated, but not firmly established. Experiments in polycythemic mice were undertaken in an attempt to separate the effect of hypoxia on iron absorption from that on the erythropoietic activity. The latter parameter was evaluated on the basis of percent values of peripheral reticulocytes, nucleated red cells in red pulp of spleen, and/or RBC-$^{59}$Fe incorporation. In the first series of experiments the erythroid response to an 18- or 96-hr period of hypoxia was selectively suppressed by administration of antierthropoietin (anti-Ep) serum. However, the enhancement of iron absorption induced by an equivalent hypoxic stimulus was not significantly modified by anti-Ep. In additional studies, polycythemic mice were subjected to either fractionated Ep administration or hypoxic stimuli, lasting 24, 48, or 72 hr. The stimulatory effect on erythropoiesis induced by Ep was either greater or equivalent to that of hypoxia. However, although these hypoxic stimuli induced a four- to sixfold increase in iron absorption, Ep administration evoked either little or no enhancement of iron absorption. These experiments provide evidence against concepts linking the primary regulation of iron absorption to the erythropoietic rate.

The mechanism(s) regulating iron absorption remain controversial despite extensive investigation. It is currently recognized that, in the presence of normal iron stores, iron absorption usually varies with the rate of erythropoiesis. However, a cause and effect relationship between these parameters has not been firmly established. In this regard, Crosby suggested that enhanced erythropoiesis led to a depletion of iron from intestinal mucosal cells which permitted increased absorption of iron. On the other hand, Mendel reported that hypoxia enhanced iron absorption in splenectomized mice in which bone marrow suppression was accomplished with large doses of radioactive strontium. However, these experiments have been criticized because the effect of the radiostrontium upon intestinal absorptive cells was not studied. The present experiments were undertaken using antierthropoietin (anti-Ep) serum in an attempt to separate the effect of hypoxia on erythropoiesis from that on iron absorption. An alternative approach involved simultaneous evaluation of both parameters following either Ep administration or hypoxia.
MATERIAL AND METHODS

Polycythemic Mice

In all experiments female mice of the CF 1 strain weighing between 20 and 25 g were main-
tained on a diet of laboratory pellets and tap water ad libitum. The animals, pretreated intra-
muscularly with 1 mg of iron-dextran on the day before initiation of the hypoxic period, were
rendered polycythemic by exposure to hypoxia (0.42 atmospheres of air) for 18 hr/day up to a
total of 230 hr. Reexposure to hypoxia or Ep administration was always initiated on day 6 post-
hypoxia, because exhypoxic polycythemic mice show complete suppression of erythropoiesis
starting at that time.7,8

Ep and Anti-Ep Serum

Step I sheep plasma Ep (0.5 IU/mg of protein) was obtained from Connaught Medical Re-
search Laboratory, Toronto, Canada. Rabbit antihuman urinary Ep was prepared according to
a previously reported procedure.9 One milliliter of this serum neutralizes at least 125 IU of
human urinary Ep or 12.5 IU of mouse Ep (Table I).

Evaluation of Iron Absorption

Eighteen-hour starved, polycythemic mice received 1 μCi 59Fe citrate (dissolved in 0.6 ml of
a 0.01 N HCl freshly prepared solution containing 7 μg of carrier iron, i.e., 36 μg of FeSO4·7H2O)
via gastric intubation by means of an 18-gauge needle. The animals were counted 2 hr and 7 days
after oral iron in a small-animal whole-body counter, which consisted of a lead castle containing
a 2 × 2-inch NaI crystal viewing a perforated, tight plastic container containing the mouse. Iron
absorption was calculated as the percentage of the final to initial counts after correction for
decay by comparison to a standard.

Evaluation of the Erythropoietic Activity

The 24- or 48-hr per cent RBC-59Fe incorporation values were employed as the basic index
of red cell production. Eighteen-hour starved, polycythemic mice received intravenously 0.5 μCi
59Fe citrate. The animals were bled by cardiac puncture 24 or 48 hr later. Blood volume was as-

Table 1. Neutralization of Ep from Either Human Urine or Mouse Serum Following
Incubation With Rabbit Antihuman Urinary Ep Serum (Anti-Ep). Assay of the
Incubation Mixtures in Exhypoxic Polycythemic Mice

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Treatment of Assay Mice</th>
<th>Mean Per Cent RBC-59Fe Incorporation ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.5 IU human urinary Ep + 0.1 ml NRS</td>
<td>22.48 ± 2.03</td>
</tr>
<tr>
<td>2</td>
<td>2.5 IU human urinary Ep + 0.1 ml anti-Ep</td>
<td>0.29 ± 0.05</td>
</tr>
<tr>
<td>3</td>
<td>12.5 IU human urinary Ep + 0.1 ml NRS</td>
<td>34.82 ± 1.64</td>
</tr>
<tr>
<td>4</td>
<td>12.5 IU human urinary Ep + 0.1 ml anti-Ep</td>
<td>0.33 ± 0.01</td>
</tr>
<tr>
<td>5</td>
<td>0.25 IU mouse serum Ep + 0.1 ml NRS</td>
<td>8.02 ± 0.59</td>
</tr>
<tr>
<td>6</td>
<td>0.25 IU mouse serum Ep + 0.1 ml anti-Ep</td>
<td>0.35 ± 0.04</td>
</tr>
<tr>
<td>7</td>
<td>1.25 IU mouse serum Ep + 0.1 ml NRS</td>
<td>17.31 ± 1.02</td>
</tr>
<tr>
<td>8</td>
<td>1.25 IU mouse serum Ep + 0.1 ml anti-Ep</td>
<td>0.41 ± 0.06</td>
</tr>
<tr>
<td>9</td>
<td>Saline + 0.1 ml NRS</td>
<td>0.37 ± 0.02</td>
</tr>
</tbody>
</table>

Anti-Ep serum was prepared according to a previously reported procedure.9 NRS, normal rabbit serum.
Human Ep was concentrated from the urine of a patient with aplastic anemia as previously described.9
Serum mouse Ep was obtained from normal mice subjected to severe hypoxia (0.37 atmospheres of air
for 8 hr). Both human and mouse Ep were previously titrated against Ep standard B (National Institute
for Medical Research, London).

The incubation of NRS or anti-Ep with either human or mouse Ep was carried out at 37°C for 30 min
in a waterbath with constant shaking. Each mouse received the amount of assay material indicated above.
The Ep activity of incubation mixtures was assayed in exhypoxic polycythemic mice according to a pre-
viously described procedure.20
sumed to be 7% of body weight. Mice with a final hematocrit of less than 56% were discarded. In experiments with anti-Ep and hypoxia, per cent values of peripheral reticulocytes and nucleated red cells in spleen red pulp were also employed as index of the erythropoietic activity.10

**Effect of Anti-Ep on Both Iron Absorption and Red Cell Production in Polycytemic Mice Subjected to Hypoxia**

In experiment 1, the mice received intraperitoneally 0.2 ml of either normal rabbit serum (NRS) or anti-Ep. The animals were subsequently subjected to a bout of hypoxia (0.42 atmosphere of air for 18 hr) starting 3 hr after the injections. Control animals dosed with 0.2 ml of NRS were maintained at room pressure. In each group (NRS + hypoxia, anti-Ep + hypoxia, NRS + room pressure) the iron absorption rate and the erythropoietic activity were evaluated simultaneously. Each group consisted of four subgroups. Two of them received radioiron orally or intravenously, either immediately or 48 hr after the end of the hypoxic stimulus. In the third and fourth subgroup, per cent values of peripheral reticulocytes or nucleated red cells in spleen red pulp were evaluated 72 or 48 hr, respectively, after the end of hypoxia. Each subgroup consisted of at least five anti-Ep-treated or eight NRS-treated mice.

In experiment 2, the mice were subjected to prolonged hypoxia (0.42 atmospheres of air for 96 hr). These animals received 4 daily injections of 0.2 ml of either NRS or anti-Ep starting immediately before exposure to hypoxia. Control mice receiving 0.2 ml/day of NRS were maintained at room pressure. As in experiment 1, two subgroups from each group received radioiron orally or intravenously, either immediately or 24 hr after the end of hypoxia, respectively. Two further subgroups were utilized as in experiment 1. The number of mice was also identical to that of experiment 1.

**Sequential Effect of Either Hypoxia or Exogenous Ep on Both Iron Absorption and Red Cell Production in Polycytemic Mice**

The animals were subjected to either subcutaneous administration of Ep (5 IU/12 hr) or a bout of hypoxia (0.42 atmospheres of air). Both Ep injections and the hypoxic stimulus lasted for either 24, 48, or 72 hr. Controls were given physiologic saline and maintained at room pressure. Radioiron was administered as usual orally or intravenously, 12 hr after the last Ep injection or immediately after the end of hypoxia, respectively. Each group consisted of at least five mice.

**RESULTS**

As indicated in Fig. 1, an 18-hr bout of hypoxia in polycytemic mice induced an impressive enhancement of the erythropoietic rate; as evaluated on the basis of radioiron incorporation values. This stimulatory effect, however, was
Fig. 2. Effect of anti-Ep administration on the erythropoietic and absorption rates in polycythemic mice subjected to a 96-hr hypoxic stimulus. Controls (C) received normal rabbit serum (NRS) and were maintained at room pressure. The second group (HYP) was injected with NRS and subjected to hypoxia (0.42 atmospheres of air for 96 hr). The third one (A-EP + HYP) received anti-Ep serum and was similarly exposed to hypoxia. Both NRS and anti-Ep were injected at 0, 24, 48, and 72 hr of the hypoxic stimulus. Radioiron incorporation values were evaluated 48 hr after $^{59}$Fe injection. Each bar represents mean ± SEM values. N.S., not significant.

completely abolished in animals primed with anti-Ep serum. It should be emphasized that further evaluation of red cell production by means of per cent values of peripheral reticulocytes or nucleated red cells in spleen yielded similar results (NRS + room pressure, 0.1 ± 0.1 (mean ± SEM) and 0.3 ± 0.1, respectively; NRS + hypoxia, 1.3 ± 0.2 and 1.5 ± 0.3; anti-Ep + hypoxia, 0.1 ± 0.1 and 0.2 ± 0.1). On the other hand, it is of relevance that the enhanced rate of iron absorption induced by hypoxia was not significantly diminished by priming with anti-Ep.

Figure 2 similarly shows that administration of anti-Ep in polycythemic mice subjected to a 96-hr period of hypoxia, although abolishing the erythroid response to the hypoxic stimulus, did not significantly modify the enhanced rate of iron absorption. In this regard, per cent values of peripheral reticulocytes and nucleated red cell in spleen red pulp were as follows: NRS + room pressure, 0.0 ± 0.0 (mean ± SEM) and 0.2 ± 0.1, respectively; NRS + hypoxia, 2.6 ± 0.5 and 4.9 ± 1.8; anti-Ep + hypoxia, 0.0 ± 0.0 and 0.1 ± 0.0.

As indicated in Fig. 3, both fractionated administration of Ep and hypoxia evoked an impressive wave of erythropoiesis in polycythemic mice. It should be noted, however, that radioiron incorporation values were significantly higher following a 24- or 48-hr period of Ep injection than those following hypoxia. On the other hand, no significant difference was observed in 72-hr studies. It is of interest that, although exposure to hypoxia for 24, 48, or 72 hr induced a four- to sixfold increase of iron absorption, only a 48-hr administration of Ep caused a barely significant rise of the absorption rate. Furthermore, no enhancement of this function was observed in mice receiving Ep for either 24 or 72 hr.
DISCUSSION

The regulation of iron absorption remains an unsolved problem. Crosby stated that a better understanding of regulatory mechanisms requires separation of the various phenomena associated with iron absorption, so that the role of each factor can be individually evaluated.

Anti-Ep serum was employed in our experiments to induce both complete and selective suppression of red cell production. Administration of anti-Ep does not modify either myelopoiesis or thrombopoiesis, but suppresses the erythropoietic activity in normal, hypophysectomized, and polycythemic rodents. The present studies in polycythemic mice showed that administration of anti-Ep abolished the erythroid response following an 18- or 96-hr exposure to hypoxia. This was not associated, however, with a significant decrease of the hypoxia-enhanced iron absorption. It was therefore concluded that changes in iron absorption following hypoxia were not dependent upon the stimulation of erythropoietic activity.

This hypothesis is strengthened by experiments involving either fractionated Ep administration or hypoxic stimuli lasting 24, 48, or 72 hr. Both Ep and hypoxia evoked an impressive wave of erythropoiesis. However, although hypoxia induced a marked enhancement of iron absorption, the stimulatory effect of Ep on this function was both temporally limited and of minor quantitative significance. This suggests that enhanced red cell production induced by hypoxia had either little or no effect upon iron absorption.

Although these studies confirm conclusions reported by Mendel, they are not liable to the same experimental criticisms. Anti-Ep exerts a more selective suppression of erythropoiesis than radiation, and the present observations include a more precise evaluation of erythropoiesis. Finally, studies involving a 96-hr hypoxic stimulus may permit insight into the mechanism of action of other stimuli (bleeding, etc.), which enhance iron absorption only after prolonged tissue hypoxia. It is therefore concluded that these experiments, in line with the clinical observations by Schiffer et al., provide strong evidence against concepts linking the primary regulation of iron absorption following hypoxia to the erythropoietic rate.

It should be noted that iron absorption is often enhanced in patients with depleted iron stores, liver cirrhosis or hemochromatosis without anemia, and compensated hemolytic states. Since these clinical conditions are not associated with tissue hypoxia, it is apparent that stimuli distinct from hypoxia may enhance iron absorption.

The present studies suggest by implication that hypoxia enhances iron absorption via either a humoral factor distinct from Ep or by a direct effect upon the intestinal mucosa. Although parabiosis experiments suggested that there was a humoral factor responsible for the regulation of iron absorption, preliminary observations in our laboratory have failed to demonstrate in hypoxic rodents a serum factor which stimulates iron absorption in recipient polycythemic mice. On the other hand, it has been reported that phenobarbital enhances iron absorption. Although Manis and Schachter presented evidence disputing this concept, the possibility should be considered that hypoxia exerts
a stimulatory effect on iron absorption via a direct influence on the intestinal mucosa.

ACKNOWLEDGMENT

We thank Mr. Antonio Di Costanzo and Mr. Biagio Ungaro for their excellent technical help.

REFERENCES

Independence of Iron Absorption From the Rate of Erythropoiesis
C. Peschle, G. P. Jori, G. Marone and M. Condorelli