Regulation of Iron Absorption and Storage Iron Turnover

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The regulation of iron supply to plasma was studied in male rats. Repeated exchange transfusions were first carried out with plasma from iron-deficient or iron-loaded animals. There was no recognizable effect on the amount of iron entering the plasma as evidenced by plasma iron concentration or iron absorption by recipient animals. In other studies, iron compounds having different tissue distribution were injected. Subsequent iron release was greater from reticuloendothelial cells than from other iron-loaded tissues. When requirements for transferrin iron were increased by exchange transfusion with high reticulocyte blood, within minutes there was a doubling of the rate of tissue iron donation. It was concluded from these studies that (1) iron turnover in the plasma is primarily determined by the number of tissue receptors for iron, particularly those of the erythron, (2) that the amount of iron supplied by each donor tissue is dependent on the output of other donor tissues, and (3) that a humoral mechanism regulating iron exchange is unlikely in view of the speed of response and magnitude of changes in plasma iron turnover. It is proposed that there is some direct mechanism that determines the movement of iron from donor tissues to unsaturated transferrin binding sites.

BODY IRON is usually maintained within relatively narrow limits. Should iron requirements for erythropoiesis increase, an increased amount of iron is provided the erythroid marrow through mobilization of storage iron and through an increase in iron absorption. Should iron ingestion be increased, an undesirable excess of iron is prevented by a reduction in the proportion absorbed. From such observations it is apparent that iron absorption and iron exchange with storage areas are regulated so as to meet the general needs of the body and, more specifically, the needs of the erythron. The manner in which this is accomplished has not been clarified. The most obvious mechanism would be within the plasma iron transport mechanism itself, but past studies have not lent support to this. A humoral mechanism of regulation has been suggested; alternately, it has been proposed that the iron content of individual tissues might in itself be a regulating factor. This study attempts to examine some of these possibilities and, on the basis of evidence obtained, to discuss the general nature of regulation.

MATERIALS AND METHODS

Male Sprague-Dawley rats 9–10 wk old, weighing 180–200 g were used. They were fed Purina Rat Chow containing 382 mg/kg of iron. Mean values for hematocrit, plasma iron, and total iron binding capacity in these animals were 42.4 ± 2.8 (1 SD),* 177 ± 58, and 508 ± 38 μg/dl plasma, respectively. In order to establish venous access, the animal was first anesthetized by an intramuscular injection of 6 μg fentanyl and 0.3 g/100 g body weight droperidol (0.015 ml/100 g body weight Inovar-vef). A PE-50 polyethylene tube (Clay Adams Division of Becton, Dickinson and Company, Parsippany, N.J.) was then inserted through the right jugular vein into the superior vena cava. The free end of the catheter was conducted subcutaneously to the back of the neck, and the lumen of the catheter was plugged with fishing line. Operative blood losses were replaced. Three to five days later, one of three general types of experiments was undertaken.

Experiment 1

In an attempt to demonstrate a humoral factor, exchange transfusions were carried out with the blood from normal rats, from iron-deficient rats, and from iron-loaded rats. The iron-deficient donor rat blood was prepared from rats fed a low-iron diet containing 5–10 mg of iron/kg from the fourth to the eighth weeks of life. Hematocrits of blood from iron-deficient animals were less than 20%, plasma iron less than 60 μg/dl, and transferrin saturation less than 10%. The blood from these animals was drawn from the abdominal aorta, and the hematocrit was normalized to 42% by removing an appropriate amount of plasma before its use. Blood was also obtained from animals placed on a low phosphate diet containing 1%–2% by weight ferrous sulfate from the sixth to the twelfth week of life so as to produce iron overload. The nonfasting transferrin saturations of animals so fed were over 75%. Donor blood from all animals was transfused within 2 hr of the time obtained. Six cycles of blood removal and blood replacement, each involving 1.5% of the animal's weight, were carried out over about 30 min. This amounted to an exchange of 9% of the animal's weight or 1.5 blood volumes.

At intervals from 2 to 63 hr after exchange, absorption from a gut loop was measured. A 20–25-cm jejunal loop was prepared in animals fasting for 24 hr, as previously described. Two milliliters of 56Fe ferrous ammonium sulfate (100 nM/ml, pH 2) was injected into the loop, and after a 60-min interval, saline equivalent to one blood volume of the animal was injected intravenously simultaneously with blood removal. The gut loop was then rapidly removed and rinsed with 20 ml of cold saline 3 times. Separate counting of the gut loop and carcass was carried out in an Armac small animal total body counter with appropriate corrections for background and geometry. Iron absorbed was expressed as:

\[ \text{Total mucosal uptake (% of dose given)} = \left( \frac{\text{Gut loop radioactivity} + \text{Carcass radioactivity}}{\text{Radioactivity given}} \right) \times 100 \]
Transfer (% of mucosal total uptake in carcass)

\[
= \frac{\text{Carcass radioactivity}}{\text{Gut loop activity + Carcass activity}} \times 100
\]

**Experiment 2**

In a second series of studies, different iron compounds were infused intravenously so as to deposit surplus iron in different storage areas. A harness was first attached to permit the connection of the caval catheter to the infusion set. A counterbalanced suspending arm was employed to keep the infusion tube out of the animal's reach, and a swivel allowed the animal to turn freely in a 25 × 30 cm cage. A total volume of 2–3 ml of various iron solutions and of saline or citrate for controls was infused by a constant rate pump (Harvard Apparatus Company, Inc., Dover, Mass.) over a 24-hr period. Absorption tests were carried out at varying times over the following 15 days as previously described.

The iron compounds employed for loading the experimental animal included rat liver ferritin, nonviable erythrocytes, and ferric citrate. Ferritin was prepared as described by Huebers. The purified ferritin was dissolved in 0.9% saline and adjusted to a final concentration of 500–800 μCi of ferritin iron/ml solution. The material was used within 2 wk. Radiolabeled ferritin was prepared from the liver of a rat given an intraperitoneal injection of 0.05-0.50 μCi 35Fe or 59Fe. The specific activity of ferritin in the standard infusion studies was 0.05 μCi 59Fe/mg. In a special study involving autoradiographs of the liver, larger amounts of radioiron were injected, and a concentration of 4 μCi 59Fe/mg was achieved. For the preparation of nonviable red cells, normal rats were bled with saline, suspended in 4 times their volume of ACD (formula B), and heated in a 40°C water bath for 20 min to prevent precipitation of hemoglobin. The blood was incubated in 100% CO2 atmosphere at room temperature for 2 hr, and then transferred to a stopper tube, centrifuged, plasma removed, and the packed cells ultrasonicated for 30 sec 3 times in a Biosonic Type IV Sonicator (Braunwell). The hemolysate was then diluted with saline to a final concentration of 3 g/100 ml of hemoglobin and centrifuged at 30,000 g for 60 min at 4°C. The supernatant was kept sterile at 4°C and used within 2 days. Immediately before use, 0.3 mg of hemoglobin was incubated at 37°C with 1 ml of freshly drawn rat plasma for 30 min in order to bind hemoglobin to haptenoglobin. This material was injected intravenously into the test animal, which was sacrificed 48 hr later. Alternatively, damaged red cells for tracer purposes were prepared as described for RE loading, except that the heating process was prolonged to 16 min. By this technique, removal of damaged cells was consistently over 90% at 3 hr and plasma hemoglobin, as detected by TCA precipitable radioactivity, was less than 2% during this period.

At the end of the iron infusion and absorption studies, some animals were sacrificed. Larger tissues labeled with 35Fe were counted in an Armac small animal total body counter with appropriate corrections for background and geometry. Aliquots of individual tissues labeled with 59Fe were also determined in a gamma-scintillation counter. When 35Fe as well as 59Fe was employed, tissues were wet ashed and were prepared for scintillation counting as described by Eakins and Brown. Appropriate corrections were made for cross-counting.

**Experiment 3**

In a third series of studies, the plasma iron turnover was determined before and after an exchange transfusion with high reticuloocyte blood. Reticulocytes were obtained by injecting donor animals once with acetylphenyl-hydrazine (50 mg/kg intraperitoneally) 6 days before. Two blood volumes (12% of body weight) were exchanged over a period of 30 min. Before the first transfusion, 0.3 μCi of 59Fe as ferrous sulfate was injected intravenously over 2 min. Over the subsequent 80 min, 5 samples of blood were drawn for analyses of plasma radioactivity and at 5, 30, and 60 min, 1.5-ml samples were drawn for plasma iron determinations. This blood sampling resulted in a decrease in hematocrit from a mean of 42.8 to 34.1. The hematocrit was returned to 42.6 ± 1.6 during the exchange transfusion of reticulocytes. After the exchange, a second dose of 59FeSO4 with 4 times the radioactivity of the first dose was injected, and 5 samples of blood were drawn over the following 30 min for determination of plasma radioactivity along with 3 samples for plasma iron determinations. The T1/2 of each plasma radioiron disappearance curve was determined by a least-square plot of the radioactivity of the 5 blood samples. The 3 plasma iron values were plotted and the extrapolated value at the T1/2 was employed in calculations. Plasma iron turnover was calculated according to the following formula:

\[
\text{Plasma iron turnover (mg/dl whole blood/day)} = \text{Pl (μg/dl) - Hct x 0.9)}
\]

\[
\text{PI (μg/dl) = (100 - Hct x 0.9)}
\]

\[
\text{Plasma iron turnover (mg/dl whole blood/day)} = \frac{\text{Pl (μg/dl) - Hct x 0.9)}}{\text{T1/2 (min)}}
\]

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absorbed iron transferred to the carcass was 35% (p = 0.08). Of two plasma volumes each (Table I), reticulocyte counts were carried out with new methylene blue, the reticulocytes in 1000 red cells being determined. Hematocrits were done by the microhematocrit technique; hemoglobin by the cyanmethemoglobin method.

### RESULTS

**Search for a Regulating Substance in Plasma**

In these studies, evidence for the presence of the regulating substance affecting iron supply was sought. Large amounts of plasma from iron-deficient and iron-loaded animals were exchange transfused, in some instances involving three exchange transfusions of two plasma volumes each (Table 1). At the time of sacrifice, both plasma iron concentration and iron absorption were examined as indicators of alterations in iron supply. The plasma iron of 6 animals receiving iron-deficient plasma averages 136 ± 13 as compared to 117 ± 13 μg/dl in 4 controls (p = 0.15). The transferrin saturation of the 2 groups of animals was 25% ± 7% and 25% ± 8%, respectively. The mean plasma iron of 4 animals receiving plasma from iron-loaded donors was 138 ± 38 as compared to 136 ± 44 in 3 controls (p = 0.95), and the respective transferrin saturations were 32% ± 7% and 28% ± 6% (p = 0.45).

Absorption measurements were carried out in the same groups of animals. Total mucosal uptake of animals given iron-deficient plasma averaged 70% ± 13% of the iron placed in the gut lumen as compared to 74% ± 15% in controls (p = 0.65). The fraction of absorbed iron transferred to the carcass was 35% ± 4% as compared to 37% ± 6% in controls (p = 0.53). Total absorption in animals given plasma from iron-loaded animals averaged 74% ± 13% as compared to 78% ± 7% in controls (p = 0.68), while the fraction transferred was 18% ± 11% versus 16% ± 4% (p = 0.72).

These studies showed no significant effect of the various plasma transfused on the amount of iron released into the blood plasma or absorbed. The somewhat greater plasma iron in animals transfused with iron-deficient plasma may well have related to the increase in total iron-binding capacity of about 75 μg/dl, since previous studies have suggested that the transferrin saturation is maintained by compensating changes in plasma iron concentration.

**The Effect of Iron Loading on Absorption**

Loading studies were carried out by the intravenous infusion of 1.25 μg of iron in the form of ferric citrate, ferritin, and nonviable red cells. The amount of iron administered was sufficient to increase the mean hepatic iron stores of 468 ± 157 μg in 20 control animals two- to threefold. The distribution of the individual iron compounds 2-3 days after the beginning of the infusion is shown in Table 2. The localization of ferritin was predominantly hepatic (84%) and autoradiographs of the liver of these animals showed the granules to be predominantly over hepatocytes (79%) and spread uniformly throughout the liver lobule. By contrast, nonviable red cells showed both splenic and hepatic activity with the later localized by autoradiography predominantly (79%) over Kupffer cells and sinusoids. The renal activity of 5% of the injected dose suggested that some intravascular hemolysis had occurred. The distribution of iron citrate was more general and also involved more loss from the

### Table 1. Effect of Plasma From Iron-Deficient and Iron-Overload Animals on Plasma Iron Concentration and Iron Absorption

<table>
<thead>
<tr>
<th>Donor Blood</th>
<th>Interval Exchange Transfusion and Absorption</th>
<th>Recipient Animals</th>
<th>Iron Absorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron Status of Donor Animal</td>
<td>Plasma Iron (μg/dl)</td>
<td>Transferrin Saturation (%)</td>
<td>#1</td>
</tr>
<tr>
<td>(A) Normal and Fe deficient</td>
<td>Normal (nonfasting)</td>
<td>145</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>Normal (fasting)</td>
<td>163</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>Iron deficient (nonfasting)</td>
<td>58</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Iron deficient (fasting)</td>
<td>54</td>
<td>7</td>
</tr>
<tr>
<td>(B) Normal and iron loaded</td>
<td>Normal (nonfasting)</td>
<td>177</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>Iron loaded (overnight fasting)</td>
<td>206</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>Iron deficient (nonfasting)</td>
<td>172-184</td>
<td>33-34</td>
</tr>
<tr>
<td></td>
<td>Iron deficient (fasting)</td>
<td>206</td>
<td>56</td>
</tr>
</tbody>
</table>

*Values at the time of the absorption measurement.
†Interval between exchanges and iron absorption (hr).
‡Values are given as mean (range).
carcass. Its distribution in the liver was predominantly parenchymal (88% by autoradiography); in contrast to the even distribution of ferritin, it was concentrated in the periportal hepatocytes. Ferric citrate also showed a greater localization in intestine and heart. The changing distribution of the injected iron over a period of 15 days was also examined (Fig. 1). Ferritin iron gradually declined from day 2 to day 10, but two-thirds of the deposited iron still remained by day 15. The same was true for the smaller hepatocytic iron load with ferric citrate. Red cell iron deposited within the reticuloendothelial cells decreased to less than 50% by day 10.

The effect on iron absorption of these 3 different compounds, each containing 1.25 mg of iron, was determined. Animals infused with 0.9% NaCl served as controls for injections of ferritin and nonviable red cells, while sodium citrate in 0.1 N HCl–0.9% NaCl served as a control for the injection of iron citrate. Total mucosal uptake after the first day was not significantly reduced with any of the compounds as compared to corresponding control values. Changes in mucosa-to-body transfer of iron are shown in Fig. 2. On day 1, a depression of iron transfer ($p = 0.005$) was observed with every compound. On day 2, absorption in animals receiving nonviable red cells and ferritin continued to be depressed ($p = 0.05$), but on days 3–5, absorption was depressed only in animals receiving nonviable red cells ($p = 0.005$ and $p = 0.05$). On day 10 and day 15, no depression of absorption was seen for any compound. Values for plasma iron and transferrin saturation from blood drawn at the time of

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Ferrous Sulfate (Tracer Dose)</th>
<th>Ferric Citrate (1.25 mg Fe)</th>
<th>Ferritin (1.25 mg Fe)</th>
<th>Nonviable Red Cells (1.25 mg Fe)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>10.5 ± 2.5</td>
<td>30.0 ± 0.8</td>
<td>84.3 ± 1.3</td>
<td>58.0 ± 1.4</td>
</tr>
<tr>
<td>Spleen</td>
<td>1.0 ± 0.0</td>
<td>0.4 ± 0.1</td>
<td>0.5 ± 0.0</td>
<td>17.0 ± 3.6</td>
</tr>
<tr>
<td>Red cells</td>
<td>63.5 ± 7.4</td>
<td>8.8 ± 1.7</td>
<td>2.5 ± 1.3</td>
<td>3.8 ± 1.0</td>
</tr>
<tr>
<td>Marrow</td>
<td>7.5 ± 2.4</td>
<td>4.3 ± 1.5</td>
<td>2.0 ± 0.8</td>
<td>4.3 ± 1.3</td>
</tr>
<tr>
<td>Total gut</td>
<td>3.8 ± 0.5</td>
<td>7.8 ± 1.0</td>
<td>1.3 ± 0.5</td>
<td>1.0 ± 0.0</td>
</tr>
<tr>
<td>Heart</td>
<td>0.4 ± 0.0</td>
<td>0.5 ± 0.1</td>
<td>0.1 ± 0.0</td>
<td>0.1 ± 3.4</td>
</tr>
<tr>
<td>Kidneys</td>
<td>1.0</td>
<td>4.0</td>
<td>0.3</td>
<td>5.3</td>
</tr>
<tr>
<td>Complete carcass</td>
<td>91.5</td>
<td>75.8</td>
<td>94.5</td>
<td>98.0</td>
</tr>
</tbody>
</table>

*Values expressed as mean and SD of the four animals in each group.

Fig. 1. Turnover of enlarged iron stores. Three groups of animals were injected with 1.25 mg of iron as ferritin, nonviable red cells, and ferric citrate. Excess iron in liver and spleen over the normal stores of 468 ± 157 mg was determined at intervals over 10 days. Ferritin-injected animals are represented by circles, nonviable red-cell-injected animals by triangles, and iron-citrate-injected animals by squares. Each point represents the average of 2 animals.

Fig. 2. Iron absorption after iron loading. On the left side is shown the amount of iron entering the plasma from the intestinal mucosa in animals who have been injected with ferritin (solid circles) and with nonviable red cells (solid triangles). The shaded areas represent the range of absorption in 5 control animals infused with saline. On the right side are shown the results of animals injected with iron citrate (solid squares) as compared to the shaded area of 5 noninjected animals. In all instances, the dose of iron injected was 1.25 mg. The vertical lines represent the range of values observed in the 2 animals studied with each type of infusion on each day.
the absorption study (Table 3), except for blood drawn immediately after the infusion, were within normal limits. In an additional experiment, the dose of iron given was lowered to 0.3 mg over 24 hr, and each compound was evaluated in 2 animals. When absorption was expressed as percent of corresponding control values, the most pronounced effect at 24 hr was seen with nonviable cells (36% ± 3% of control values) as compared to ferric citrate (55% ± 24%) and ferritin (75% ± 22%).

The effect of reticuloendothelial iron loading on release of hepatocyte iron and the effect of hepatocyte iron loading on RE release was also examined. In each study six animals were infused with 1.25 mg of iron in the form of nonviable red cells or ferric citrate, respectively. One, two, or three days later, two of these animals were injected with a tracer, labeling the opposite compartment, i.e., 0.3 mg hemoglobin-^{59}Fe as hemoglobin-haptoglobin complex to label hepatocyte iron or nonviable ^{59}Fe tagged red cells to label RE iron. Two days after the injection, ^{59}Fe red cell incorporation into red cells was measured (Fig. 3). After reticuloendothelial preloading the hepatocyte trace release was consistently only about half as large as from nonloaded control animals, p < 0.005, whereas hepatocyte preloading did not appear to affect the subsequent RE release.

**The Effect of Reticulocyte Transfusion on Tissue Iron Release**

Baseline plasma iron turnover was determined for 11 normal animals and was repeated 40 min after the beginning of the exchange transfusion with high reticulocyte blood (Table 4). The exchange transfusion itself took 30 min. At the end of the first plasma iron turnover, circulating reticulocytes were 8.4% ± 2.2%.

Table 3. Plasma Iron and Transferrin Saturation and Controls and Animals (1.25 mg Iron Injected)

<table>
<thead>
<tr>
<th>Days After Start of Infusion</th>
<th>Saline Control (n = 4)</th>
<th>Nonviable Red Cells (1.25 mg Fe, n = 2)</th>
<th>Ferritin* (1.25 mg Fe, n = 2)</th>
<th>Citrate Control (n = 2)</th>
<th>Ferric Citrate (1.25 mg Fe, n = 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PI (µg/dl) TS (%)</td>
<td>PI (µg/dl) TS (%)</td>
<td>PI (µg/dl) TS (%)</td>
<td>PI (µg/dl) TS (%)</td>
<td>PI (µg/dl) TS (%)</td>
</tr>
<tr>
<td>1</td>
<td>94 ± 16 22 ± 7</td>
<td>147 ± 38</td>
<td>1674 ± 20</td>
<td>111 ± 20</td>
<td>234 ± 59</td>
</tr>
<tr>
<td>2</td>
<td>112 ± 31 28 ± 10</td>
<td>85 ± 22</td>
<td>141 ± 23</td>
<td>112 ± 24</td>
<td>2208 ± 24</td>
</tr>
<tr>
<td>3</td>
<td>125 ± 16 28 ± 4</td>
<td>128 ± 43</td>
<td>157 ± 37</td>
<td>131 ± 34</td>
<td>233 ± 28</td>
</tr>
<tr>
<td>5</td>
<td>119 ± 19 30 ± 6</td>
<td>81 ± 16</td>
<td>167 ± 39</td>
<td>148 ± 32</td>
<td>155 ± 40</td>
</tr>
<tr>
<td>10</td>
<td>102 ± 13 26 ± 5</td>
<td>74 ± 21</td>
<td>103 ± 26</td>
<td>136 ± 29</td>
<td>156 ± 29</td>
</tr>
<tr>
<td>15</td>
<td>96 ± 35 24 ± 8</td>
<td>157 ± 36</td>
<td>97 ± 22</td>
<td>115 ± 21</td>
<td>132 ± 33</td>
</tr>
</tbody>
</table>

*Plasma iron values do not differentiate transferrin-bound and ferritin-bound iron.

After the reticulocyte exchange transfusion and the second radioiron turnover, reticulocytes were elevated to 30.9% ± 3.8%. The mean T/2 of the first turnover was 55.8 ± 16.6 min, while the mean T/2 of the second turnover was 19.2 ± 3.3 min. The mean plasma iron value at the T/2 for the first turnover was 167 ± 72, while the mean plasma iron for the second turnover was 133 ± 32 µg/dl plasma. The rate of change of plasma iron during the first turnover was −0.28 µg and for the second turnover −0.39 µg iron/dl plasma/min. The mean value for the initial plasma iron turnover was 2.02 mg/dl whole blood/day, of which 1.76 came from tissues. This is to be contrasted to the turnover of 4.26 for the second turnover with 3.85
Table 4. The Effect of Reticulocyte Transfusion on Plasma Iron Turnover

<table>
<thead>
<tr>
<th>Studies</th>
<th>Initial PIT</th>
<th>Posttransfusion PIT</th>
<th>Tissue Increment PIT T2-PIT, Studies</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plasma Iron Turnover (mg/dl Whole Blood/Day)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Initial</td>
<td>After Transfusion</td>
<td>Total From Tissues</td>
<td>Total From Tissues</td>
</tr>
<tr>
<td>1</td>
<td>65</td>
<td>117</td>
<td>1.06 ± 0.53</td>
<td>2.07 ± 0.49</td>
</tr>
<tr>
<td>2</td>
<td>146</td>
<td>145</td>
<td>2.20 ± 0.53</td>
<td>3.29 ± 1.02</td>
</tr>
<tr>
<td>3</td>
<td>140</td>
<td>116</td>
<td>1.84 ± 0.53</td>
<td>3.37 ± 1.02</td>
</tr>
<tr>
<td>4</td>
<td>222</td>
<td>165</td>
<td>2.46 ± 1.82</td>
<td>5.16 ± 1.11</td>
</tr>
<tr>
<td>5</td>
<td>81</td>
<td>78</td>
<td>1.21 ± 0.53</td>
<td>2.44 ± 1.02</td>
</tr>
<tr>
<td>6</td>
<td>321</td>
<td>149</td>
<td>2.37 ± 1.62</td>
<td>5.32 ± 1.02</td>
</tr>
<tr>
<td>7</td>
<td>145</td>
<td>99</td>
<td>2.35 ± 1.64</td>
<td>3.76 ± 1.11</td>
</tr>
<tr>
<td>8</td>
<td>144</td>
<td>152</td>
<td>1.73 ± 1.93</td>
<td>4.75 ± 1.50</td>
</tr>
<tr>
<td>9</td>
<td>162</td>
<td>120</td>
<td>2.49 ± 2.82</td>
<td>4.40 ± 1.02</td>
</tr>
<tr>
<td>10</td>
<td>250</td>
<td>186</td>
<td>2.44 ± 1.90</td>
<td>6.07 ± 1.02</td>
</tr>
</tbody>
</table>

μg/dl whole blood/day coming from tissues. Thus, the increase in tissue release of iron during the second turnover was 2.09 mg/kg/day or an increase of 119% over the first turnover (Table 4).

DISCUSSION

Iron exchange is mediated by the plasma protein, transferrin, which receives iron from iron-donating tissues and releases it to other tissues to meet their iron requirements or to permit storage of excess iron. The plasma iron concentration reflects the balance between input and output, whereas the plasma iron turnover indicates the amount of iron exchanged. Contributions of individual tissues may also be examined by isotopic techniques. All of these methods were employed in the present studies.

Body iron is regulated within relatively narrow limits through variations in absorption and through mobilization or deposition of storage iron. Furthermore, the plasma iron pool, despite its small size, meets widely varying needs with little variation in iron concentration. Thus, a normal plasma iron is usually seen with hemolytic anemia where the total turnover may be increased four to five times and in hypoproliferative anemia where red cell production may be decreased to half normal. The manner in which such a close coordination between iron supply and tissues is achieved has not been understood.

The most logical mechanism of control is through changes in transferrin saturation. According to this hypothesis, an increase in iron removal would result in a decrease in plasma iron and an increase in unsaturated iron binding sites on transferrin; this in turn would cause an increased removal of iron from iron donor sites. In the other direction, a decrease in tissue uptake would cause a greater saturation of circulating transferrin with iron and less iron would enter the plasma from tissues. Various attempts to identify changes in transferrin saturation leading to changes in iron turnover have been unsuccessful. Indeed, recent studies of the relation between plasma iron and plasma iron turnover have shown the reverse, i.e., an increase in plasma iron resulting in an increase in turnover and a decrease in a decreased plasma iron turnover.

Furthermore, the injection of unsaturated transferrin so as to create additional unsaturated binding sites does not increase absorption from the gastrointestinal (GI) tract. Likewise, the intravenous injection of iron so as to raise transferrin saturation does not affect the amount of iron absorbed, although it does affect its internal distribution. Clinical observations are also inconsistent with the original hypothesis. The excessive iron absorption in thalassemia and idiopathic hemochromatosis occurs even though the plasma iron is elevated and the transferrin saturated. In these conditions the reticuloendothelial cell also discharges catabolized red cell iron rather than storing it, despite the high plasma iron and transferrin saturation. Such studies would seem to indicate that neither the level of plasma iron nor the unsaturated iron-binding capacity regulates iron supply.

Other humoral factors have been considered as possible regulators. Because of the relation of iron absorption to erythropoiesis, erythropoietin has been considered but did not appear to affect absorption as long as erythropoiesis was not altered. While serum ferritin has not been directly tested, the intravenous injection of tissue ferritin had no effect. Clinical observations also are against the importance of serum ferritin, since extremely high ferritin levels are found with increased absorption in idiopathic hemochromatosis and thalassemia, whereas low levels of ferritin in iron deficiency are also associated with high absorption.

More general assays have been carried out in which plasma from iron-deficient and iron-loaded animals has been injected and effects on absorption sought. In some studies, positive effects have been reported, i.e., a
The concept of an integrated supply of iron from various tissues may be further extended to consider that the contribution of each might depend on iron needs and on the iron content of the donor cell. 

Conrad and Crosby have suggested that absorption is dependent on the mucosal iron content of individual cells, this being determined not only by luminal iron but also by transferrin iron entering the mucosal cell from the inside. Subsequent studies have provided variable data concerning the relation between iron content of mucosal cells and their absorptive behavior. It seems likely that the importance of cellular iron content cannot be answered until more is known concerning the molecular machinery involved in iron transport and may depend on analysis of a specific subcellular fraction.

A difficulty in examining regulation has been the time required to produce alterations in iron exchange. It has not been clear whether this was due to the time required for change in tissue needs or that time was required for a change in the iron supply system. By introducing reticulocytes into circulation, it was found possible within 15 min to greatly increase tissue receptors for transferrin and to place them in optimal contact with transport iron. Surprisingly, this was not associated with any appreciable decrease in plasma iron or increase in unsaturated iron-binding capacity, but was associated with a doubling of the tissue supply of iron. In the setting of this study, it seemed unlikely there would be other complicating factors, such as increased erythropoietin or hypoxia.

The results obtained in this study are consistent with the earlier suggestion of Cavill and associates of a labile pool of iron in all body tissues and of the interaction of various donor tissues in supplying iron requirements. These studies do show that tissue need is the primary determinant of plasma iron turnover and that an easily mobilized pool of iron enters circulation when needed. This establishes the presence of some rapidly responsive supply process not dependent on transferrin saturation per se.

REFERENCES


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Regulation of iron absorption and storage iron turnover

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