Regulation of Iron Absorption and Storage Iron Turnover

By Amadeus Rosenmund, Stephen Gerber, Helmut Huebers, and Clement Finch

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

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*Variations in this article are expressed as standard deviation.

From the University of Washington, Department of Medicine, Division of Hematology, Seattle, Wash.

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Address reprint requests to Dr. Clement Finch, University of Washington, School of Medicine, Department of Medicine, Division of Hematology Rm-10, Seattle, Wash. 98195.

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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 nCi/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:

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)

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

**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 x 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 μg of ferritin iron/ml solution. The material was used within 2 wk. Radioactive ferritin was prepared from the liver of a rat given an intraperitoneal injection of 0.05-0.50 μCi 57Fe or 59Fe. The specific activity of ferritin in the standard infusion studies was 0.05 μCi 57Fe/mg. In a special study involving autoradiographs of the liver, larger amounts of radioiron were injected, and a concentration of 4 μCi 57Fe/mg was achieved. For the preparation of nonviable red cells, normal rats were bled repeatedly by cardiac puncture one-third to one-sixth of their blood volumes and then injected in the tail vein with 5-10 mCi of 57Fe or 59Fe ferrous sulfate (pH 2.0) over a period of 2 wk. Subsequent injections of radioiron were given every 2-3 wk to maintain activity and to provide uniform tagging of the red cell population. These donor animals were used as red cell donors beginning 6 wk after the initial injection of radioiron. Plasma was removed after centrifugation at 500 g at 4°C for 10 min. Packed cells were washed 3 times in cold saline, suspended in 4 times their volume of ACD (formula B), and heated in a 40°C water bath for 20 mm to prevent precipitation of hemoglobin. The blood was then transferred to a stopper tube, centrifuged, plasma removed, and the packed cells ultrasonicated for 30 sec 3 times in a Bionetics 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 haptoglobin. This material was injected intravenously into the test animal, which was sacrificed 48 hr later. Alternately, 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 57Fe were counted in an Amray small animal total body counter with appropriate corrections for background and geometry. Aliquots of individual tissues labeled with 57Fe were also determined in a gamma-scintillation counter. When 59Fe as well as 57Fe 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 57Fe 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 ± 2.8 to 34 ± 1.1. The hematocrit was returned to 42.6 ± 1.6 during the exchange transfusion of reticulocytes. After the exchange, a dose of 57FeSO4 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 T½ 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 T½ was employed in calculations. Plasma iron turnover was calculated according to the following formula:

\[
\text{Plasma iron turnover (mg/dl whole blood/day)} = \frac{\text{PI (μg/dl) } (100 - \text{Hct} \times 0.9)}{\text{T½ (min)} } \times 100
\]

The rate of change of plasma iron per hour was also determined. From this, the amount of iron donated by 100 ml of whole blood per 24 hr (PID) was calculated according to the formula:

\[
\text{Plasma iron change (mg/100 ml whole blood/day)} = \frac{\text{μg/hr} \times 24 \times (100 - \text{Hct} \times 0.9) }{100}
\]

The amount of iron derived from tissues was calculated by subtracting the plasma iron decrease from the plasma iron turnover.

Plasma iron was determined according to the procedure described by the International Standardization Committee and total iron

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binding capacity by the method of Cook et al.17 Chemical determinations for tissue iron were carried out on wet ashed tissues, employing bathophenanthroline as a color indicator. 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 plasmas 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.28

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>
<thead>
<tr>
<th>Iron Status of Donor Animal</th>
<th>Plasma Iron (μg/dl)</th>
<th>Transferrin Saturation (%)</th>
<th>No.</th>
<th>Plasma Iron* (μg/dl)</th>
<th>Transferrin Saturation* (%)</th>
<th>Total Iron Absorption†</th>
<th>Iron Absorption†</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) Normal and Fe deficient</td>
<td>Normal (nonfasting)</td>
<td>145</td>
<td>21</td>
<td>2</td>
<td>123</td>
<td>31</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>(nonfasting)</td>
<td>163</td>
<td>34</td>
<td>2</td>
<td>112</td>
<td>(17,21)</td>
<td>(72.94)</td>
</tr>
<tr>
<td></td>
<td>Iron deficient</td>
<td>58</td>
<td>18</td>
<td>1</td>
<td>125</td>
<td>(17,21)</td>
<td>(65.67)</td>
</tr>
<tr>
<td></td>
<td>(nonfasting)</td>
<td>54</td>
<td>7</td>
<td>1</td>
<td>(114-140)</td>
<td>(18-33)</td>
<td>(45-86)</td>
</tr>
<tr>
<td>(B) Normal and iron loaded</td>
<td>Normal (nonfasting)</td>
<td>177</td>
<td>34</td>
<td>3</td>
<td>136</td>
<td>(126-168)</td>
<td>(63-79)</td>
</tr>
<tr>
<td></td>
<td>Iron loaded (overnight fasting)</td>
<td>206</td>
<td>56</td>
<td>4</td>
<td>136</td>
<td>(95-168)</td>
<td>(70-81)</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 peniportal 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 \( (p = 0.005 \text{ and } p = 0.05) \). 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

![EXCESS IRON IN LIVER AND SPLEEN (mg)]

**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.

![IRON ABSORPTION](#)

**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.

### Table 2. Tissue Distribution of Iron-Loading Compounds (% of Injected Radioiron)

<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</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</td>
</tr>
<tr>
<td>Heart</td>
<td>0.4 ± 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 ± 0</td>
<td>4.0 ± 0.0</td>
<td>0.3 ± 0.0</td>
<td>5.3</td>
</tr>
<tr>
<td>Complete carcass</td>
<td>91.5 ± 7.4</td>
<td>75.8 ± 1.3</td>
<td>94.5 ± 3.6</td>
<td>98.0</td>
</tr>
</tbody>
</table>

*Values expressed as mean and SD of the four animals in each group.*
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 of hemoglobin-59Fe as hemoglobin-haptoglobin complex to label hepatocyte iron or nonviable 59Fe tagged red cells to label RE iron. Two days after the injection, 59Fe 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%, After the reticulocyte exchange transfusion and the second radioiron turnover, reticulocytes were elevated to 30.9% ± 3.8%. The mean T½ of the first turnover was 55.8 ± 16.6 min, while the mean T½ of the second turnover was 19.2 ± 3.3 min. The mean plasma iron value at the T½ 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 3. Plasma Iron and Transferrin Saturation and Controls and Animals (1.25 mg Iron Injected)

<table>
<thead>
<tr>
<th>Days After Infusion of I.25 mg Iron</th>
<th>Saline Control (n = 2)</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>Start of Infusion</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 ± 111 20</td>
<td>234 ± 59</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>112 ± 31 28 ± 10</td>
<td>85 ± 22</td>
<td>141 ± 112 23</td>
<td>84 ± 20</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>125 ± 16 30 ± 4</td>
<td>128 ± 43</td>
<td>157 ± 131 34</td>
<td>181 ± 29</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>119 ± 19 30 ± 6</td>
<td>81 ± 16</td>
<td>103 ± 136 29</td>
<td>136 ± 29</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>102 ± 13 26 ± 5</td>
<td>74 ± 21</td>
<td>97 ± 115 21</td>
<td>132 ± 33</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>96 ± 35 24 ± 8</td>
<td>157 ± 36</td>
<td>96 ± 140 32</td>
<td>108 ± 32</td>
<td></td>
</tr>
</tbody>
</table>

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

Fig. 3. Iron store mobilization. On the left side, hepatocyte radioiron release is examined after reticuloendothelial loading with 1.25 mg of iron as nonviable red cells (solid circles). This is compared to hepatocyte radioiron release of a control animal who had not been injected with excess iron (open circles). Hepatocyte iron was labeled by the injection of radioiron-labeled hemoglobin bound to haptoglobin. On the right side, reticuloendothelial iron release is examined after hepatocyte loading by ferritin injection. Nonviable red cells labeled with radioiron were used to tag reticuloendothelial iron stores. Results in iron-loaded animals are shown by the solid triangles, while results in animals not loaded with iron are shown by the open triangles. Each point represents the average of 2 animals while vertical lines show the range of values.
Table 4. The Effect of Reticulocyte Transfusion on Plasma Iron Turnover

<table>
<thead>
<tr>
<th>Plasma Iron Turnover (mg/dl Whole Blood/Day)</th>
<th>Plasma Iron Turnover (mg/dl Plasma)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial PIT</td>
<td>Total From Tissues</td>
</tr>
<tr>
<td>Initial Transfusion</td>
<td>Total</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>1</td>
<td>65</td>
</tr>
<tr>
<td>2</td>
<td>146</td>
</tr>
<tr>
<td>3</td>
<td>140</td>
</tr>
<tr>
<td>4</td>
<td>222</td>
</tr>
<tr>
<td>5</td>
<td>81</td>
</tr>
<tr>
<td>6</td>
<td>321</td>
</tr>
<tr>
<td>7</td>
<td>145</td>
</tr>
<tr>
<td>8</td>
<td>162</td>
</tr>
<tr>
<td>9</td>
<td>250</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>167 ± 72</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 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. However, other investigators have been unable to demonstrate effects of injected plasma at time intervals up to 3 or 4 days. Our preliminary attempts at plasma injections were similarly unsuccessful. But a possible weakness of such observations was thought to be the amount of plasma injected. Accordingly, exchange transfusions were employed. In studies carried out 18 hr after the last exchange, there was no evidence of any increase in iron entering the plasma as monitored by the amount of plasma iron or the amount of iron absorbed. Since large and repeated plasma exchanges were carried out over intervals of up to 3 days without effect, it seemed unlikely that there was a humoral factor present capable of altering iron supply by donating tissues.

One of the most conspicuous determinants of iron absorption is the state of body iron stores. For example, high absorption is found in subjects whose red cell values are quite normal but whose stores are altered. This relationship was further explored by loading different tissues with iron: the RE system by injecting nonviable cells, the hepatocytes with ferritin, and parenchymal tissues in general with ferrous ammonium sulfate. Our study showed a temporary effect on absorption whenever iron was introduced. The greatest and most protracted effect, however, was found with reticuloendothelial loading. Parenteral iron has previously been observed to suppress absorption, and this was ascribed to mucosal loading with iron from the plasma. However, in the current studies, the effect of iron citrate, which produced the most mucosal cell loading, was no greater and certainly less prolonged than that of nonviable red cells. It seemed likely that the amount of iron provided by each iron-donating tissue is dependent on the amount provided by other tissues. The greater amount of iron released from reticuloendothelial cells after iron loading presumably accounted for the greater suppression of absorption and hepatic iron release.

REFERENCES

Regulation of iron absorption and storage iron turnover
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