The Significance of Iron Turnover in the Control of Iron Absorption

By LEWIS R. WEINTRAUB, MARCEL E. CONRAD AND WILLIAM H. CROSBY

With the technical assistance of Arthur L. Foy

The stimulus to gastrointestinal absorption of iron remains unknown. Previous work seems to indicate that hepatic iron and total body iron stores are not as important as the rate of iron utilization in regulating iron absorption. Stimulation of erythropoiesis by blood letting increases iron utilization. However, following acute phlebotomy there is a 4- to 5-day delay before iron absorption is increased. The present study was undertaken to delineate the plasma iron kinetics and intestinal iron content during this critical 5-day period in order to further our understanding of the regulation of intestinal absorption of iron.

METHODS

Ten to 15 μc. of Fe⁵⁹ citrate (specific activity 1.5–5 μc./μg. iron) were incubated for 30 minutes with a quantity of the subject's own plasma sufficient to bind the available iron. A gravimetric aliquot was injected intravenously and six to eight samples of blood were obtained with heparinized syringes during the following 1½ to 2 hours. Two ml. aliquots of plasma were counted for 10,000 counts in a well type 2-inch NaI crystal scintillation detector (Packard Auto Gamma Spectrometer, Model 410 A). The plasma Fe⁵⁹ T½ was determined from the first exponential function of a three-pool model system. Plasma iron turnover was calculated as described by Huff et al. Plasma iron and total iron binding capacity were determined by the Ramsay methods. All plasma iron turnover studies were performed and blood for plasma iron determinations obtained from normal volunteers in a fasting state at 8 a.m.

Male albino rats (W.R.C.F. strain) weighing 250–300 Gm. were used in the animal experiments. They were fed a standard rat and mouse diet (G. L. Baking Co., Frederick, Md.), and it was estimated that each rat ingested 2 mg. of iron per day. The principles of laboratory animal care as promulgated by the National Society for Medical Research were observed. The test dose for iron absorption studies was 0.5 μc. ferrous⁵⁹ citrate/0.5 mg. ferrous sulfate 0.5 ml. of distilled water administered through an olive-tipped 17-gauge endoesophageal needle to rats fasted for 16 hours. Whole body radioactivity (0.8 mνv* to ∞) was measured in a small animal whole body liquid scintillation detector (Packard ARMAC) 3 hours and 7 days after dosing to determine the percentage of the dose absorbed. The reliability of this technic has been reported previously.

The non-heme iron content of the first quarter of the small intestine was determined after a 16-hour fast. The gut segment was removed and cleaned of mesenteric fat. The specimen was then opened and washed free of intestinal contents with iron-free saline. The tissue was prepared for chemical analysis in a tissue homogenizer (Virtis) and the non-heme iron content was determined by the method of Bruckmann and Zondek. Rats were bled by the insertion of a heparinized capillary tube into the retro-orbital plexus. Transfusion of homologous whole blood was performed through a polyethylene catheter (#50) inserted in the external jugular vein of the rat.
RESULTS

I. Plasma Iron Kinetics

Eight healthy male volunteers (age 19–25) with normal hemoglobins, red cell indices and plasma iron values were selected for this study. There was no previous history of blood donations. A preliminary plasma iron turnover was performed, followed by removal of 500 ml. of whole blood. Thereafter, serial specimens were obtained for plasma iron determinations. One determination of plasma iron turnover was made in each individual on the 1st, 3rd, 5th or 14th day following phlebotomy.

Figure 1 shows the trend in plasma iron in five individuals. This is representative of the entire group. The plasma iron remained normal until the 4th or 5th day. This was followed by a transient decrease in each subject, and the values returned to normal levels by the 11th day. There was no significant change in the plasma iron kinetics on the 1st or 3rd day (table 1). However, on the 5th day, associated with the decrease in plasma iron, there was an increased rate of clearance of radioactive iron (lowered T½). This compensatory change maintained the plasma iron turnover close to the pre-phlebotomy level. In the subjects tested on the 14th day, the plasma iron had returned to normal. The Fe59 T½ remained shortened; thus the plasma iron turnover was significantly increased.

II. Iron Content of the Small Intestine

To ascertain if the content of iron in the small intestine changed during the first 5 days following acute blood loss, we turned to animal experiments. Animals were bled 5 ml. of whole blood and iron absorption studies were performed on days one through five. Figure 2 demonstrates the presence of a 4- to 5-day lag period before iron absorption was significantly increased in the entire group. This phenomenon is similar to that demonstrated in humans and dogs. A similar group of rats was bled the same quantity of blood and killed at daily intervals for the determination of intestinal content of non-heme iron. A decrease in the concentration of iron in the gut was noted at the time of increased intestinal absorption of iron (fig. 3).
IRON TURNOVER IN THE CONTROL OF IRON ABSORPTION

Table 1.—Iron Kinetics Following Phlebotomy

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An attempt was made to inhibit marrow hyperplasia by transfusion of blood at intervals following phlebotomy. Rats were bled 5 ml. of whole blood. The same quantity was transfused at 6 hours or at 1, 2, 3, or 4 days following the phlebotomy. An iron absorption study was performed on the 5th day post-phlebotomy. It was demonstrated that the absorption of iron remained normal if the blood was replaced within 3 days (fig. 4).

DISCUSSION

Following an acute decrease in the circulating red cell mass there was a lag of 4 to 5 days before we demonstrated a change in the plasma iron kinetics. The shortened Fe^59 T½ and simultaneous fall in plasma iron suggested a shift of iron from the labile plasma pool into the bone marrow. Each subject was assumed to have an adequate iron store since he had normal hematologic values, plasma iron and iron binding capacity, as well as a negative history of blood donations or gastrointestinal blood loss. We believe that in normal individuals, iron stores are relatively stable, as has been demonstrated by others. Thus, during mobilization of iron to meet the immediate demands of the bone marrow’s increasing activity, there was a transient fall in the plasma iron. In the animals, associated in time with the above findings, there was a significant fall in the non-heme iron concentration of the small intestine. From the data we infer that the intestinal iron pool is in a labile relationship with the plasma iron kinetics. In support of this hypothesis, we have demonstrated a 38 per cent fall in the concentration of iron in the small intestine of iron-loaded animals following acute blood loss which occurred despite the presence of an extra body iron store 5 times greater than the quantity removed at the time of phlebotomy.
We have also demonstrated an increase in the intestinal content of iron following an intravenous dose of iron.\textsuperscript{14} Radioautographs showed the iron to be localized in the epithelial cells. At the time of maximal gut concentration of the parenterally administered iron, there was a decrease in the intestinal absorption of an oral dose of radioactive iron. Contrawise, in the present study we have shown a significant reduction in the iron content of the gut associated with an increase in iron absorption. Thus, the content of iron in
the intestine seems to be important in the regulation of the epithelial cell’s ability to absorb iron. The amount of iron in the gut in turn appears to be related to the utilization or plasma clearance or iron. This would explain why human subjects 14 days post-phlebotomy demonstrated an increased iron absorption with a normal plasma iron.15

The regulation of iron absorption following blood loss may be explained in the following manner. As the marrow activity increases, there is a change in the plasma iron turnover. This in turn preferentially reduces the intestinal content of iron and gut absorption is increased. The iron stores, as in the liver, are relatively stable and slowly engage into this system. If, following phlebotomy, the equivalent amount of blood is replaced before the marrow hypertrophies, the turnover of iron will not increase and iron absorption remains normal.

**SUMMARY**

Following acute blood loss, there is a 4- to 5-day lag before any demonstrable change in plasma iron kinetics. Then there is a shortened Fe¹⁵⁰ T½ and transient fall in the plasma iron. Associated with this is a decrease in the intestinal content of iron and an increased absorption of iron. We suggest the iron content of the epithelial cell is important in the regulation of its ability to absorb iron. The intestinal iron content is in turn closely controlled by the plasma iron turnover.

**SUMMARIO IN INTERLINGUA**

Post acute perditas de sanguine, il occurre un retardo de 4 a 5 dies ante que alterat’ones in le ferrocinetica del plasma deveni demonstrabile. Postea il occurre un reduction del tempore de medie valor pro Fe¹⁵⁰ e un transiente
declino in le ferro del plasma. Associate con isto es un declino in le conten
to intestinal de ferro e un augmentate absorption de ferro. Nos presenta
le theses que le contenuto de ferro de cellula epithelial es importante in le
regulation de su capacitate de absorber ferro. Le contenuto intestinal de
ferro, de su parte, es intimemente dependente del metabolismo de ferro
in le plasma.

REFERENCES
stores in the control of iron absorp-
2. Hahn, P. F., Bale, W. F., Ross, J. F.,
Balfour, W. M., and Whipple, G. H.: 
Radioactive iron absorption by gastro-
intestinal tract. Influence of anemia,
anoxia and antecedent feeding. Distri-
bution in growing dogs. J. Exper.
Med. 78:169, 1943.
3. Bothwell, T. H., Pirzio-Biroli, G., and
Finch, C. A.: Iron absorption. I. Fac-
tors influencing absorption. J. Lab.
4. Sharney, L., Schwartz, L., Wasserman,
L. R., Port, S., and Leavitt, D.: Pool
systems in iron metabolism; With spe-
cial reference to polycythemia vera. 
Proc. Soc. Exper. Biol. & Med. 87: 
489, 1954.
5. Hsu, R. L., Hennessy, T. G., Austin, B.
E., Garcia, J. F., Roberts, B. M., and
Lawrence, J. H.: Plasma and red cell
iron turnover in normal subjects and 
in patients having various hemato-
poietic disorders. J. Clin. Invest. 29: 
1041, 1950.
6. Ramsay, W. N. M.: The determina-
tion of iron in blood, plasma or serum. 
7. —: The determination of the total iron
8. Forrester, R. H., Conrad, M. E., and
Crosby, W. H.: Measurement of total
body iron59 in animals using whole-
body liquid scintillation detectors. 
Proc. Soc. Exper. Biol. & Med. 111:
improved method for the determina-
tion of non-hemin iron. J. Biol. Chem.
135:23, 1940.
11. Finch, C. A.: Body iron exchange in 
Fe59 oxide in red cell formation. J.
13. Pollycove, M., and Mortimer, L.: The 
quantitative determination of iron 
kinetcs and hemaglobin synthesis in 
human subjects. J. Clin. Invest. 40:
753, 1961.
14. Conrad, M. E., Weintraub, L. R., and
Crosby, W. H.: The role of the small 
intestine in iron kinetics. J. Clin. In-
vest. In press.
15. Forrester, R. H., Conrad, M. E., and
Crosby, W. H.: Unpublished observa-
tions.

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