The Gastrointestinal Tract and Iron Absorption

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With the technical assistance of Betty Merrill and Natalie Lawson

It has been demonstrated repeatedly that intestinal absorption of iron is responsive to the body's needs; enhanced absorption being found with increased need, and diminished absorption with decreased need. Thus there is agreement that intestinal iron absorption is regulated, but there is no general agreement as to the nature of the regulatory mechanism. As part of all previous hypotheses on the mechanism of control, two assumptions had been held:

1. The amount of iron which entered the intestinal epithelial cells was believed to participate in the control of subsequent iron absorption.

2. All iron entering intestinal epithelial cells was absorbed into blood.

Studies reported by Conrad et al. suggested that the second assumption was not valid. These studies were done by giving Fe59 intragastrically to rats which were later killed at varying intervals. Four hours after the animals were dosed, radio-autographs of the small intestine showed the entire villus to be labeled, and over the next 24 hours, progression of label toward villous tip was seen. These investigators postulated that iron held in this way by epithelial mucosal cells might subsequently be absorbed into blood or, as movement of the label indicated, might be sloughed into the lumen with the epithelial cell at the end of its life span. No data were presented regarding how much iron this might represent or if it constituted a significant proportion of iron entering intestinal epithelial cells. If it did, another concept of iron absorption had to be considered, i.e., unregulated entry of iron into intestinal epithelial cells with the controlling mechanism regulating only subsequent movement from epithelial cells to blood. The present report attempts to add further information along these lines.

Materials and Methods

General Method

Male albino rats of the Walter Reed-Carworth Farm strain of the Wistar, weighing approximately 400 gm., were used for the experiments. The method for determining iron absorption using Fe59 and a whole-body liquid scintillation counter has been described in detail. The test dose of iron consisted of 0.2-0.3 μc of Fe59 as citrate in 0.25 ml of water with various carrier doses of ferrous sulfate. All but the iron-deficient rats were fed ad libitum a balanced diet (Walter Reed Rat and Mouse Diet). The rat's daily intake of iron on this diet is 1000 to 1500 μg. Iron deficiency was produced in some by repeated bleeding and by feeding a milk-powder, iron-poor diet. The series of four bleedings totalling 12 ml. of blood was begun 8 weeks before study and ended 4 days before.

Iron loading was produced 4 weeks prior to study with an intramuscular injection of 15 mg. of iron as an iron-dextran complex (Imferon). Forty-eight hours prior to most of the...
THE G.I. TRACT AND IRON ABSORPTION

Table 1.—Distribution of Radioiron 2 Hours after Gastric Administration

<table>
<thead>
<tr>
<th>Rat</th>
<th>Stomach</th>
<th>Upper 1/4</th>
<th>2nd 1/4</th>
<th>3rd 1/4</th>
<th>4th 1/4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.0</td>
<td>68.0</td>
<td>8.4</td>
<td>7.8</td>
<td>14.8</td>
</tr>
<tr>
<td>2</td>
<td>1.7</td>
<td>67.2</td>
<td>13.6</td>
<td>10.5</td>
<td>7.0</td>
</tr>
<tr>
<td>3</td>
<td>0.8</td>
<td>74.5</td>
<td>13.9</td>
<td>6.6</td>
<td>4.2</td>
</tr>
</tbody>
</table>

studies, the animals were placed on the iron-poor diet. All food was removed 16 hours preceding study and only water was allowed. Four to 6 hours following dosing in those studies not terminated by that time, the iron-poor diet was put back into the cages. Dosing was accomplished by gastric intubation with an olive-tipped 17-gauge endoesophageal tube under very light ether anesthesia. At the designated time after dosing, preselected animals were killed by a sharp blow on the head. The abdomen was opened immediately, a firm tie placed around the lower esophagus to prevent reflux, and the entire gastrointestinal tract up to the ileocecal junction quickly removed. At the same time the entire large intestine including the anus was dissected free and in most instances discarded. Stomach and small intestine were opened lengthwise and washed 5 times in 30 ml. of 1 per cent calcium EDTA to remove unabsorbed iron. Since the fourth and fifth washes contained less than 0.5 per cent of administered radioactivity, the remaining iron was considered to be fixed to cells. For counting, the washed stomach and small intestine were placed into a 250 ml. plastic bottle which was then filled with water. Radioactivity of carcass and gut were determined separately in the whole-body counter. The validity of counting the gut in this way was established by first counting in the described manner. As a check on counting geometry, the gut subsequently was digested completely in acid, the digestate washed into a 250 ml. plastic bottle which was brought up to volume and recounted. No difference in count was found. In all studies, sufficient counts were obtained to give a counting error of approximately one per cent.

EXPERIMENTAL STUDIES AND RESULTS

Gastric and Small Intestinal Distribution of Radioiron Given Intragastrically

Study 1: To determine the gastrointestinal distribution of radioiron 2 hours after intragastric administration, three normal rats were given 250 μg. of Fe⁵⁹-tagged iron. Two hours later they were killed and handled as described. The small intestine was divided into fourths before counting.

Results are shown in table 1. The upper one-fourth of the small intestine contained 67.2 to 74.5 per cent of the retained iron. Less than 2 per cent was found in stomach.

Gastrointestinal Distribution of Radioiron Given Intravenously

Study 2: To determine the distribution in the gastrointestinal tract of Fe⁵⁹ injected intravenously, 16 normal rats were given into a tail vein, 0.25 μc. of Fe⁵⁹ citrate containing 0.02 μg. of iron. After a preset time, they were killed and handled as for the absorption studies except that washing of the gut was not done. Stomach, small intestine, and colon were counted separately.

Table 2 shows the results. Six hours or more after injection, stomach and colon were found to contain less iron than small intestine. The relatively small proportion of injected radioactivity present in the small intestine indi-
Table 2.—Gastrointestinal Distribution of Radioiron at Varying Intervals after Intravenous Injection into Normal Rats

<table>
<thead>
<tr>
<th>Time Killed after Dosing Hours</th>
<th>Percentage of Injected Radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stomach</td>
</tr>
<tr>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>6</td>
<td>—</td>
</tr>
<tr>
<td>6</td>
<td>—</td>
</tr>
<tr>
<td>24</td>
<td>—</td>
</tr>
<tr>
<td>24</td>
<td>—</td>
</tr>
<tr>
<td>24</td>
<td>0.3†</td>
</tr>
<tr>
<td>48</td>
<td>0.3</td>
</tr>
<tr>
<td>48</td>
<td>0.6</td>
</tr>
</tbody>
</table>

*Includes stomach.
†Mean of four rats. Range 0.2-0.6 per cent.
‡Mean of eight rats. Range 1.6-4.5 per cent.

cated that recirculation to intestine of absorbed radioiron would not greatly influence the intestinal retention patterns observed in normal rats in Study 3.

Absorption Pattern

Study 3: The pattern of iron absorption was studied in 36 normal rats given 250 µg. of Fe<sup>59</sup>-tagged iron and killed from 15 minutes to 51 hours after dosing. Each group contained either three or four rats. All animals were given iron by gastric intubation except the 15-minute group in which it was put via

![Pattern of iron absorption into carcass of normal rats following a single 250 µg. dose of Fe<sup>59</sup>-tagged iron. Each block represents the mean of either three or four rats. Brackets include the range of values.](image URL)
Fig. 2.—Pattern of intestinal iron uptake and retention in normal rats following a single 250 μg. dose of Fe⁵⁹-tagged iron. Each block represents the mean of either three or four rats. Brackets include the range of values.

a catheter directly into duodenum. These rats were anesthetized with Nembutal intraperitoneally, the abdomen opened, a polyethylene catheter inserted per os and manually guided through the pylorus into duodenum. The catheter was removed after dosing. At each time, radioiron content of carcass and gut was determined separately.

Figure 1 shows the general pattern of absorption into carcass. There was an early phase of rapid absorption which lasted up to 1 to 2 hours followed by a phase of slower absorption which extended up to 12 to 24 hours. In figure 2 the pattern of intestinal iron uptake and retention is shown. Peak intestinal iron content was found 30 minutes after dosing. As time elapsed a progressive disappearance of intestinal iron was noted.

Effect of Body Iron Stores on Pattern of Intestinal Iron Retention

Study 4: To determine the effect of body iron stores on the pattern of intestinal iron retention, 25 normal, 16 iron-deficient and 12 iron-loaded rats were given 250 μg. of Fe⁵⁹-tagged iron. The animals were killed at varying intervals after dosing and iron retained by the washed small intestine was determined.

Figures 3, 4 and 5 show the patterns for normal, iron-loaded, and iron-deficient rats respectively. In the iron-loaded group there appeared to be less uptake of iron by the intestine and a somewhat slower loss of retained iron compared to the normal and iron-deficient groups. There are multiple physiologic processes underlying these retention patterns and, in addition, the variation was such that for the number of animals studied, only a general, overall comparison could be made.
Comparison of Absorption and Intestinal Uptake at 2 Hours with 8-Day Absorption—Effect of Body Iron Stores

Study 5: Iron absorption by 2 hours was compared with final absorption at 8 days and in addition the effect of body iron stores on absorption was determined. For this study 40 rats were randomized into four equal groups. One group was given 15 mg. of parenteral iron to produce iron overload (15 FeL), a second was given 2 mg. to insure adequate iron stores (2 FeL), a third group was untreated (normal) and a fourth was made iron deficient (FeD) as
IRON DEFICIENT RATS
INTESTINAL RETENTION
250 µg. dose

Fig. 5.—Pattern of intestinal iron retention in iron-deficient rats following a single 250 µg. dose of Fe\textsuperscript{59}-tagged iron. Each block represents the mean of four rats. Time is drawn to scale. Brackets include one standard error.

described. Each group of 10 rats was randomized further into a 2-hour and an 8-day group, each containing five rats. The 2-hour group was killed 2 hours after the oral dose of 250 µg. of iron and the carcass and gut counted separately as described. The remaining five were counted 8 days after dosing when all unabsorbed iron had been excreted. After being counted the rats were killed and the livers removed for chemical determination of total iron content.\textsuperscript{140}

The relationship between mean absorption into carcass by 2 hours and final mean absorption at 8 days is shown in figure 6 and table 3. Two-hour absorption was 82 per cent of the 8-day absorption in group 15 Fel, 78 per cent in group 2 FeL, 64 per cent in the normal, and 75 per cent in group FeD. These results indicated that most of the iron absorbed following a single dose took place within 2 hours. A clear distinction could be made at 2 hours and at 8 days between the decreased absorption of the excessively iron-loaded group (15 FeL), the markedly increased absorption of the iron-deficient (FeD), and normal absorption (normal and 2 FeL).

When combined carcass-plus-gut iron content at 2 hours was compared to final absorption at 8 days, it was evident in all groups except the iron deficient that more iron had been taken up by intestine than was ultimately absorbed into carcass. (fig. 7, table 3). Assuming that final absorption of rats killed at 2 hours would have been similar to mean absorption found at 8 days, an estimate of subsequent loss of intestinal iron retained at 2 hours was: 15 FeL, 82 per cent; 2 FeL, 78 per cent; normal, 64 per cent. This was approximately 11 µg. of iron for each of the three groups and, when compared to mean final absorption at 8 days, represented a sizable loss (table 3). For group 15 FeL,

\*Performed by Pearl Anderson, Ph.D., Department of Hematology, Walter Reed Army Institute of Research.
the loss was greater than mean absorption at 8 days, 11.1 compared to 8.3 μg.; for group 2 FeL, 11.7 compared to 17.1 μg.; and for the normal group, 11.6 compared to 20.1 μg. The relationship at 2 hours between iron content of gut and carcass is shown in figure 8 and table 3. In all but the iron-deficient group, more iron was retained by intestine than had been moved into carcass.

When 2-hour, carcass-plus-gut iron content in all four groups was compared, group 15 FeL showed the least and group FeD the greatest (fig. 7, table 3). An a priori hypothesis had been that there would not be a significant difference between 2-hour carcass-plus-gut content in group 15 FeL and the normal group. This hypothesis was rejected since the difference was highly significant (p < .01). Since peak intestinal iron uptake had been achieved by 2 hours, and since any iron taken up was either in carcass or retained by intestine, this result indicated that there was a difference between these two groups in the amount of iron passing from lumen into intestinal epithelial cells. An obviously greater difference was seen between these two groups and group FeD (fig. 7, table 3).

Results of chemical iron analyses of livers removed from all 8-day groups are shown in table 4. It is of interest that the increase produced by injecting 2 mg. of iron was not sufficient to cause a decrease in iron absorption.

Effect of Body Iron Stores and Iron Dosage on Total Uptake at 2 Hours versus Final Absorption

Study 6: The combined effect of body iron stores and iron content of test dose on the relationship of 2-hour to final absorption was determined. Ten
normal rats were given 25 μg. and 10 iron-loaded rats 12.5 μg. of Fe⁵⁹-tagged iron. One-half of each group was killed after 2 hours and radioiron content of carcass and gut was determined. Final absorption was determined when the remaining rats were killed 48 hours after dosing. Radioactivity of the washed gut was added to carcass for calculation of total absorption at 48 hours.

The results show that when the dose of iron given to normal rats was decreased to 25 μg., mean carcass-plus-gut iron content at 2 hours was approximately the same as mean final absorption (fig. 9). This was different from that found with the 250 μg. dose (fig. 7). At this lower dose, normal rats showed a pattern similar to iron-deficient rats given 250 μg. of iron (fig. 7). When 12.5 μg. was given to iron-loaded rats (fig. 9), the relationship between the 2-hour value (carcass plus gut) and final absorption was almost identical to iron-loaded rats given 250 μg. of iron (fig. 7). With this lower dose, 88 per cent of iron retained by intestine at 2 hours was subsequently lost. This meant loss of approximately 2 μg. of iron compared to final absorption of 2.5 μg.

Fig. 7.—Relationship between combined carcass-plus-gut iron content at 2 hours and final absorption at 8 days. Each block represents the mean of five rats. Brackets include one standard error. Key to abbreviations: FeL is iron loaded with 2 or 15 mg. Fe; FeD is iron deficient.
These studies showed that in rats given intragastrically a single dose of an iron salt, approximately 60 to 80 per cent of total absorption took place within the first 2 hours. The remainder occurred at a much slower rate over the subsequent 12 to 20 hours. A similar pattern has been found in humans, dogs, guinea pigs, and rats. What terminates the early phase of rapid absorption is not known but several important factors have been found. One is the formation of nonabsorbable iron complexes in the intestinal lumen. Duthie and co-workers found that little of the iron remaining in dog duodenal loops after 2 hours was in an absorbable form. Pirzio-Biroli found that iron added to duodenal secretions from iron-loaded mice was not absorbed as well as when normal or iron-deficient secretions were used. Other studies on the role of intestinal secretions and bile showed little effect on iron absorption. Although 85 per cent of iron added in vitro was bound by normal rat intestinal secretions, consisting mainly of mucous, subsequent absorption of this iron was not significantly different from iron in a saline solution. The difference

Table 4.—Chemical Iron Analyses (Study 5)

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean Body Weight (Gm.)</th>
<th>Mean Liver Weight (Gm.)</th>
<th>Mean Liver Iron (mg. Fe ± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FeD</td>
<td>413</td>
<td>13.233</td>
<td>0.38 ± 0.033</td>
</tr>
<tr>
<td>N</td>
<td>401</td>
<td>13.552</td>
<td>1.74 ± 0.22</td>
</tr>
<tr>
<td>2 FeL</td>
<td>407</td>
<td>13.842</td>
<td>2.65 ± 0.19</td>
</tr>
<tr>
<td>15 FeL</td>
<td>393</td>
<td>14.291</td>
<td>4.24 ± 0.11</td>
</tr>
</tbody>
</table>
in results could be due either to the amount of iron used or to binding of iron in a different manner under in vivo conditions in the intestinal lumen.

A second important factor is transit of iron from an area of highly efficient iron absorption (duodenum) to a much less efficient area (jejunum). It has been shown in rats that the first 6 to 8 cm. of small intestine are capable of more rapid and efficient iron absorption than the remainder of the small intestine.\textsuperscript{22}

It was postulated that the phase of rapid absorption was terminated as a result of increasing saturation of the iron binding protein,\textsuperscript{12} but subsequent studies\textsuperscript{24} make this seem unlikely.

Our results did not clarify why the phase of rapid absorption ended but they showed a great difference in the amount of iron moved into carcass during this period when iron-loaded, normal, and iron-deficient rats were compared; the ratio observed was 1:2:14, respectively. Furthermore, the results showed that following a dose of 250 \(\mu\)g., the early phase ended despite retention of a significant amount of iron in the intestinal wall. This suggested that either a carrier system was "exhausted" or, more likely, that in addition to a rapid transport system another mechanism existed for putting iron into a more stable, slowly released form. Granick postulated a dual pathway for movement of iron through the mucosal cells and believed the slowly released form of iron to be ferritin.\textsuperscript{24} He found increased amounts of ferritin in intestinal mucosa of guinea pigs 4 hours after they were fed large amounts of iron.\textsuperscript{9}

Brown and Rother were unable to find ferritin in rat intestinal mucosa during iron absorption.\textsuperscript{17} However, they recovered iron in two forms: either associated with amino acids, glycine and serine, a form they considered to be undergoing rapid transit to blood; or in slowly released form bound to a protein apparently distinct from ferritin. Our results did not establish the nature of
the temporarily stored iron but they were in agreement with the concept of a dual pathway for iron in intestinal mucosal cells. The relationship between size of administered dose to capacity of the rapid transport mechanism seemed to determine the quantity of iron put into slowly released storage form. For example, if the amount of iron given was large in relation to the capacity for rapid transport (normal rats given 250 μg. dose, 15 FeL given 12.5 to 250 μg.), a significant proportion of iron was put into stable form; but if the amount of administered iron was small in relation to the capacity for rapid transport (iron deficient rats given 250 μg., normal rats given 25 μg.), the amount of iron put into stable form was relatively small. This relationship was very important since in whatever form mucosal cells held iron, normal and iron-loaded rats were unable effectively to release and transfer the iron to blood. As a result, there was loss of a significant amount of iron back into lumen as mucosal cells were sloughed at the end of their life span. This phenomenon was first pointed out by Conrad and co-workers based on their radio-autographic findings. In our studies, peak intestinal uptake occurred 30 to 60 minutes after iron was administered and subsequently a gradual decrease was observed. The pattern of disappearance represented a complex phenomenon with several probable underlying processes in addition to sloughing of mucosal cells. These were:

1. Continued uptake of iron from lumen.
2. Dwindling of the dose of iron in the intestinal lumen.
3. Recirculation to intestine of absorbed radiiron. The intravenous iron distribution studies in normal rats showed that this would not greatly influence the pattern.
4. Transfer of iron from mucosal cells to blood.

A possibility which was considered and excluded was that iron entered mucosal cells in an unregulated manner with the amount absorbed being controlled solely at the level of transfer from mucosal cells to blood. Our results showed a significant difference in the amount of iron entering mucosal cells when iron-loaded, normal, and iron-deficient rats were compared. From an identical test dose the smallest amount entered the mucosal cells of iron-loaded animals and the greatest amount the iron-deficient. This meant that two steps were involved in the regulation of iron absorption:

1. Movement of iron from lumen into mucosal cells.
2. Movement of iron from mucosal cells to blood.

This two-step concept for iron absorption has been considered previously, based on studies in humans, dogs, and rats. When iron absorption in normal rats was compared to enhanced (iron-deficient) and depressed (iron-loaded) iron absorption, both steps were either increased or decreased respectively. The predominant effect, however, appeared to be on the second step, movement of iron from mucosal cells to blood. Similar results were found in studies done with rat intestinal loops both in vitro and in vivo. Whether the regulation was intrinsic to mucosal cells could not be determined from our studies.

**SUMMARY**

1. Using Fe and whole body counting, various aspects of gastrointestinal
absorption of iron salts were studied in normal, iron-loaded, and iron-deficient rats.

2. Following a single intragastric dose of iron, peak small intestinal uptake was observed by 30 to 60 minutes with subsequent gradual loss over 24-hour period.

3. Iron absorption showed two phases, an initial period of rapid absorption lasting up to 2 hours during which 60 to 80 per cent of total absorption into carcass took place. The remainder took place at a slow rate over the subsequent 12 to 20 hours.

4. Depending on the relationship between body iron stores and dose of administered iron, the intestine may take up more iron than is ultimately transferred to plasma. A variable amount of this iron is lost when the epithelial cell is sloughed into the gastrointestinal lumen.

5. Regulation of iron absorption appears to involve two steps, mucosal uptake and transfer to the blood.

SUMMARY IN INTERLINGUA

1. Fe\textsuperscript{51} e contation in sanguine total eseva usate in le studio de vane aspectos del absorption gastrointestinal de sales de ferro in rattos normal, rattos cargate de ferro, e rattos in carentia de ferro.

2. Post le administration intragastric de unic doses de ferro, maximos de absorption per le intestino tenue eseva observate intra 30 a 60 minutas, con gradual perditas subsequente in le curso de 24 horas.

3. Le absorption de ferro monstrava duo phases. Durante le prime, de circa 2 horas, un rapide absorption ad in le corpore occurreva, amontante a inter 60 e 80 pro cento del total. Le resto eseva absorbite plus lentemente in le curso del secunde phase de 12 a 20 horas additional.

4. In dependentia del relation inter le reservas corporee de ferro e le dose de ferro administrate, le intestino pote acceptar plus ferro que lo que es transferite ultimemente ad in le plasma. Un variabile quantitate de iste ferro es perdite quando le cellula epithelial se decade ad in le lumine gastrointestinal.

5. Il pare que le regulation del absorption de ferro ha a facer con duo stadios: le acceptance mucosal e le transferimento ad in le sanguine.

REFERENCES


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