Hepatic Iron Deposition in Humans

I. First-Pass Hepatic Deposition of Intestinally Absorbed Iron in Patients with Low Plasma Latent Iron-Binding Capacity

By R. A. Fawwaz, H. S. Winchell, M. Pollycove and T. Sargent

Wheby and Jones demonstrated that rats, whose plasma iron-binding capacity was saturated by an acute intravenous infusion of iron, deposited a large fraction of intestinally absorbed iron in the liver during the first pass of portal venous blood through this organ. Subsequently, Wheby and Umpiere demonstrated similar results in normal human subjects given sufficient iron to acutely saturate their plasma iron-binding capacity. Our results in patients with spontaneous chronic low plasma latent iron-binding capacity agree with those obtained by Wheby and Umpiere.

The present results were obtained using a new triple radioiron-isotope technic which allows for simultaneous measurement of the fraction of orally administered iron absorbed into the body during a 2-week period, the fraction of orally administered iron appearing in the systemic circulation over a 6-hour period, the fraction of orally administered iron absorbed and deposited in the liver during this 6-hour period, and the fraction of iron initially deposited in the liver and then released into the systemic circulation during the subsequent 2 weeks. This technic further allows for visualization of the distribution of iron in the intestinal tract and the remainder of the body while intestinal absorption is occurring.

Materials and Methods

Intestinal iron absorption was studied in four normal subjects and six patients with low plasma latent iron-binding capacity: three with hemochromatosis (studied over 3 months after cessation of phlebotomy therapy), two with sideroblastic anemia, and one with porphyria cutanea tarda. The kinetics of intestinal iron absorption was studied by oral administration of 40 μc of Fe⁵² and 1 μc of Fe⁹⁴ in 4-mg. carrier ferrous sulfate, following an 8-hour fast. Concurrent with the administration of the above isotopes, plasma iron turnover was measured by administering intravenously 60 μc of Fe⁵¹ as ferrous ammonium citrate (specific activity 1 μc per 1 μg.) which had been incubated for one-half hour with 10 ml. of autologous plasma.

Plasma samples for radioiron activity were obtained at 15- to 30-minute intervals for the
Fig. 1.—Correlation of the location of the Fe\textsuperscript{52} in the gastrointestinal tract with its rate of absorption into the plasma at various times after its oral administration to a normal subject (H.B.). The per cent Fe\textsuperscript{52} absorbed and appearing in the systemic plasma during ½-hour intervals is plotted above each photoscan. The shaded areas of the graphs represent the time at which the corresponding photoscan was taken. Note that no radioiron is detectable in the liver area at any time during the study.

Radioiron absorption from the gastrointestinal tract following its oral administration was calculated by three separate methods: (1) The per cent of orally administered Fe\textsuperscript{52} absorbed into the body over a 14-day period was calculated utilizing the whole-body counter.\textsuperscript{4} (2) The per cent of orally administered Fe\textsuperscript{52} absorbed as calculated by red cell incorporation at the fourteenth day was determined according to the method of Saybor and Finch.\textsuperscript{5} (3) The rate at which orally administered Fe\textsuperscript{52} appeared in the systemic circulation over a 6-hour period was calculated using the method of Halberg and Soble.\textsuperscript{6} The total amount of iron absorbed and appearing in the systemic circulation was taken as the integrated rate of absorption over this time interval.

The Fe\textsuperscript{52} activity was separated from the Fe\textsuperscript{55} activity by immediate counting of the plasma samples using a thallium-activated sodium iodide, well-type scintillation counter, and similarly recounting them after 7 days, at which time the Fe\textsuperscript{55} (t\textsubscript{1/2}=8.2 hours) had decayed to a negligible level. Fe\textsuperscript{55} plasma and red-cell activity was determined by counting the samples in a gas-flow counter under beryllium filters and correcting for activity due to Fe\textsuperscript{59}.\textsuperscript{7}

The distribution of orally administered Fe\textsuperscript{52} in the subject was visualized throughout the study by using the Anger positron camera.\textsuperscript{8} Semiquantitative estimates of the Fe\textsuperscript{52} deposition in the liver were obtained from external coincidence counting rates over the liver, expressed as a fraction of the total external coincidence counting rate of the entire Fe\textsuperscript{52} bolus.

In one patient (P.G.) with a plasma latent iron-binding capacity of 9 \( \mu \)g. per 100 ml., the plasma volume determined using \textsuperscript{131}I albumin was compared with that determined in
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Table 1.—Summary of Results of Iron-Absorption Studies Obtained on Four Normal Subjects and Six Patients with Low Plasma Latent Iron-Binding Capacity

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Hemoglobin concentration Gm./100 ml.</th>
<th>Fasting serum iron μg/100 ml.</th>
<th>Fasting latent iron binding capacity μg/100 ml.</th>
<th>% Fe(^{59}) absorbed and incorporated into red cells at 2 weeks (1)</th>
<th>% Fe(^{59}) absorbed and appearing in the systemic plasma over 6 hours (3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S.A.</td>
<td>14.2</td>
<td>116</td>
<td>295</td>
<td>12.0</td>
<td>Not done</td>
</tr>
<tr>
<td>H.B.</td>
<td>12.9</td>
<td>126</td>
<td>227</td>
<td>28.9</td>
<td>26.2</td>
</tr>
<tr>
<td>M.S.</td>
<td>13.4</td>
<td>72</td>
<td>216</td>
<td>7.5</td>
<td>8.0</td>
</tr>
<tr>
<td>J.B.</td>
<td>14.8</td>
<td>130</td>
<td>308</td>
<td>16.0</td>
<td>Not done</td>
</tr>
<tr>
<td>Hemochromatosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A.F.</td>
<td>15.0</td>
<td>210</td>
<td>15</td>
<td>73.0</td>
<td>8.2</td>
</tr>
<tr>
<td>P.C.</td>
<td>12.9</td>
<td>229</td>
<td>9</td>
<td>29.0</td>
<td>Not done</td>
</tr>
<tr>
<td>J.R.</td>
<td>14.4</td>
<td>193</td>
<td>46</td>
<td>56.0</td>
<td>6.2</td>
</tr>
<tr>
<td>Porphyria Cutanea Tarda</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A.E.</td>
<td>14.4</td>
<td>230</td>
<td>13</td>
<td>21.8</td>
<td>1.2</td>
</tr>
<tr>
<td>Sideroblastic Anemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S.C.</td>
<td>11.0</td>
<td>290</td>
<td>20</td>
<td>12.0</td>
<td>Not done</td>
</tr>
<tr>
<td>C.S.</td>
<td>10.0</td>
<td>160</td>
<td>66</td>
<td>45.0</td>
<td>5.0</td>
</tr>
</tbody>
</table>

Note that in patients with low plasma latent iron-binding capacity there is a discrepancy between the per cent radioiron absorbed into the body at 2 weeks (whole-body counter) and the per cent radioiron absorbed as calculated by red-cell incorporation at the fourteenth day. In these patients there also is an abnormal discrepancy between the per cent radioiron absorbed into the body at 2 weeks and the per cent radioiron absorbed and appearing in the systemic plasma during the first 6 hours. (H.B., P.C., and S.C. are female; the remaining subjects are male.)

two different fashions using Fe\(^{59}\). In this patient radionuclides were used to determine plasma volumes by the following methods: (1) One μc. of Fe\(^{59}\) as ferrous ammonium citrate, specific activity 10 μc. per 1 μg., and 10 μc. of I\(^{131}\) albumin were simultaneously incubated for one-half hour with 250 ml. of the patient’s blood and reinjected intravenously. (2) One μc. of Fe\(^{59}\) as ferrous ammonium citrate, specific activity 10 μc. per 1 μg., and 10 μc. of I\(^{131}\) albumin were simultaneously incubated with 5 ml. of normal donor plasma and injected intravenously.

Hemoglobin concentration, serum iron, and plasma latent iron-binding capacity were determined on the initial blood sample of each patient. Serum iron was determined by the method of Peters,

\(^8\) while the plasma latent iron-binding capacity was determined by the method of Tauxe.

\(^9\) All patients had plasma volumes determined with I\(^{131}\) albumin.

RESULTS

Figure 1 correlates the location of the Fe\(^{57}\) in the gastrointestinal tract with its rate of absorption into the plasma at various times after its oral administration to a normal subject (H.B.). It is seen that there is no detectable radioiron over the liver area.

Close agreement was obtained in the two normal subjects in whom the iron absorption calculated using total body retention of Fe\(^{59}\) (whole-body counter) was compared with that calculated using double isotope incorporation into red cells (Table 1). In the four normal subjects, between 40 to 80 per cent of the total radioiron absorbed appeared in the systemic plasma during the first 6 hours of the study.
Fig. 2.—Correlation of the location of the Fe$^{52}$ in the gastrointestinal tract with its rate of absorption into the plasma at various times after its oral administration to a patient (A.F.) with hemochromatosis and low plasma latent iron-binding capacity. The per cent Fe$^{52}$ absorbed into the plasma during half-hour intervals is plotted above each photoscan. The shaded areas of the graphs represent the time at which the corresponding photoscan was obtained. Note the early and significant radioiron deposition in the liver.

A discrepancy was obtained in the four patients having low plasma latent iron-binding capacity in whom the iron absorption calculated using total body retention of Fe$^{59}$ (whole-body counter) was compared to that calculated using double isotope incorporation into red cells. The iron absorption calculated by double isotope incorporation into red cells was less than 12 per cent of that calculated by total body retention of Fe$^{59}$. In the six patients with low plasma latent iron-binding capacity, less than 16 per cent of the total radioiron absorbed appeared in the systemic plasma during the first 6 hours of the study.

Figure 2 correlates the location of Fe$^{52}$ in the gastrointestinal tract with its rate of absorption into the systemic plasma at various times after its oral administration to a patient (A.F.) with hemochromatosis and a low plasma latent iron-binding capacity. This study was performed 10 months after an initial course of 120 phlebotomies. It can be seen that within 2 hours after the initiation of the study considerable orally administered Fe$^{52}$ was deposited in the liver. At 6 hours, 8.2 per cent of the radioiron was absorbed and appeared in the systemic plasma and an estimated 40 per cent of the ingested Fe$^{52}$ was deposited in the liver. Thus, four-fifths of the radioiron absorbed from the gastrointestinal tract during the first 6 hours was deposited
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Fig. 3.—Total body scans obtained 5 hours after an oral dose of Fe\(^{52}\) in a patient with hemochromatosis (A.F.). The scan on the left was obtained subsequent to an initial course of phlebotomy at a time when his plasma latent iron-binding capacity was 15 \(\mu\)g. per cent. The scan on the right was obtained 4 months after a second course of phlebotomy at which time his plasma latent iron-binding capacity was 180 \(\mu\)g. per cent. In both instances total body absorption of iron (whole-body counter) was 73 per cent of the orally administered 4 mg. dose. Note the deposition of radioiron in the liver when the latent iron-binding capacity is low, and its absence from the liver when the latent iron-binding capacity is normal.

In the liver. Similar results were obtained in the five other patients with low plasma latent iron-binding capacity.

Patient (A.F.) was studied again 3 months after completion of a second course of 10 phlebotomies. His plasma latent iron-binding capacity at this time was 180 \(\mu\)g. per 100 ml. Figure 3 compares the photoscans taken following an oral radioiron dose administered at this time (scan on the right) as compared with the initial study when the plasma latent iron-binding capacity was 15 \(\mu\)g. per 100 ml. (scan on the left). It can be seen that after 5 hours of radioiron ingestion a considerable amount was deposited in the liver when the plasma latent iron-binding capacity was low, but none was detectable in the liver when the plasma latent iron-binding capacity was normal.

Figure 4 represents two different plasma radioiron clearance studies performed on patient P.C., a hemochromatotic with a plasma latent iron-binding capacity of 9 \(\mu\)g. per 100 ml. The ordinate is expressed as the per cent of the administered dose present in the plasma and is calculated on the assumption that the \(\text{I}^{131}\) albumin plasma volume represents the true plasma volume. When the radioiron was incubated with normal donor plasma prior to its intravenous administration, its initial distribution volume was identical to that obtained with \(\text{I}^{131}\) albumin. However, a rapid initial phase of plasma radio-
Fig. 4.—Clearance of radionuclides from the plasma in a patient (P.G.) with hemochromatosis and a low plasma latent iron-binding capacity (9 μg. per 100 ml.) following the intravenous administration of (1) 10 ml. of the patient’s plasma incubated for ½ hour with $^{131}$I-albumin, (2) 5 ml. of donor plasma (normal plasma latent iron-binding capacity) incubated for ½ hour with $^{59}$Fe ferrous ammonium citrate (0.1 μg. carrier ferrous ammonium citrate), and (3) 250 ml. of the patient’s blood incubated for ¾ hour with $^{59}$Fe ferrous ammonium citrate (0.1 μg. carrier ferrous ammonium citrate.)

iron clearance with a resulting spuriously low zero-time extrapolate was obtained when radioiron was previously incubated with 250 ml. of autologous blood. In each instance the slope of the plasma radioiron clearance after 15 minutes was consistent with removal of transferrin-bound radio-iron from the plasma.

DISCUSSION

Our results in the patients with spontaneous chronic low plasma latent iron-binding capacity were similar to those obtained by Wheby and Umpiere in normal human subjects in whom the plasma transferrin was acutely saturated. As shown in Table 1, in our patients with low plasma latent iron-binding capacity, the fraction of orally administered iron absorbed into the body, calculated utilizing double isotope incorporation into red cells, was much lower than that calculated utilizing total body retention of radioiron (whole-body counter). Correspondingly, the fraction of orally administered iron absorbed and appearing in the systemic plasma during the initial 6-hour period was also abnormally low in patients with low plasma latent iron-binding capacity. Clearly, in these patients a large fraction of the iron absorbed from the gastrointestinal tract into the portal vein did not reach the systemic circulation. Photoscans of patients with low plasma latent iron-
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binding capacity demonstrated that there was significant deposition of iron in the liver within 2 hours of its oral administration (Fig. 2). At 6 hours an estimated four-fifths of the total iron absorbed from the gastrointestinal tract is deposited in the liver. Thus, in these patients with spontaneous chronic low plasma latent iron-binding capacity, a major fraction of the iron absorbed from the gastrointestinal tract into the portal vein was deposited in the liver without prior appearance in the systemic circulation. Moreover, such hepatic iron was not released to any considerable extent during the subsequent 2 weeks, as evidenced by the marked discrepancy between calculations of total radioiron absorption at 2 weeks, measured by the whole-body counter, and the iron absorption measured by double isotope incorporation into red cells 2 weeks after the initiation of the study (Table 1).

That this initial rapid radioiron deposition in the liver is related to the transferrin saturation with iron is demonstrated in patient (A.F.) with hemochromatosis. This patient showed the characteristic hepatic iron deposition within 2 hours after its oral administration at a time when the plasma latent iron-binding capacity was low, but hepatic iron was not detectable when the plasma latent iron-binding capacity was normal (Fig. 3).

To elucidate the mechanism by which intestinally absorbed iron is deposited in the liver in patients with low plasma latent iron-binding capacity, studies were performed on hepatic uptake of intravenously administered radioiron. In a hemochromatotic patient (PG) with a plasma iron-binding capacity of 9 μg per 100 ml., the initial distribution volume of radioiron incubated with normal donor plasma agreed well with the initial distribution volume (plasma volume) of 125I albumin (Fig. 4), indicating no initial rapid component of hepatic iron deposition. However, in studies on this patient where radioiron was administered intravenously as ferrous ammonium citrate subsequent to incubation with 250 ml. of autologous blood initial rapid clearance of radioiron from the plasma was noted and an abnormally high distribution volume was obtained (Fig. 4). In both studies the rate of plasma clearance of radioiron after 15 minutes was consistent with the slow removal of transferrin-bound radioiron from the plasma (Fig. 4). These results suggest that in this patient with low plasma iron-binding capacity, iron incubated with a large volume of autologous plasma existed in two forms when it reached the liver: (1) bound to transferrin in a normal fashion, resulting in a subsequent, relatively slow rate of plasma clearance of such iron; and (2) either not bound to transferrin or abnormally bound, resulting in a relatively rapid initial plasma clearance of such iron consequent to its rapid deposition in the liver.

As previously proposed by Wheby and Jones and Wheby and Umpiere, we may explain our intestinal absorption results in patients with spontaneous chronic low latent iron-binding capacity by postulating that iron absorbed from the gastrointestinal tract is not initially bound to transferrin. When the plasma latent iron-binding capacity is low, much of the intestinally absorbed iron either does not become bound to transferrin, or is abnormally bound, and is removed by the liver on the first pass of portal venous blood through this organ.
SUMMARY

In patients with spontaneous chronic low plasma latent iron-binding capacity, a large fraction of the intestinally absorbed iron is deposited in the liver without prior appearance in the systemic plasma. Such hepatic iron is not released to any large extent during the subsequent 2 weeks.

Our results are consistent with the hypothesis that iron absorbed from the intestinal tract is not initially bound to transferrin. When the plasma latent iron-binding capacity is normal, iron binds to transferrin prior to reaching the liver; when the plasma latent iron-binding capacity is low, a portion of the iron reaches the liver either unbound or abnormally bound to transferrin, resulting in its immediate hepatic deposition.

SUMMARIO IN INTERLINGUA

In patientes con spontane- e chronicamente basse latente capacitae ferro-ligatori in le plasma, un grande fraction del intestinalmente absorbite ferro es deponite in le hepate sin apparer previemente in le plasma del circulation major. Iste depositos, hepatic de ferro non es liberate a grados notable in le curso del sequente duo septimanas.

Nostre resultatos congrue con le hypothese que ferro absorbite ab le vias intestinal non es ligate initialmente a transferrina. Quando le capacitae del plasma a ligar ferro latente es normal, ferro se liga con transferrina ante que illo arriva in le hepate. Quando ille capacitae es debile, un portion del ferro arriva in le hepate in stato non ligate o anormalmente ligate a transferrina, resultante in un immediate deposition hepatic.

REFERENCES

Hepatic Iron Deposition in Humans: I. First-Pass Hepatic Deposition of Intestinally Absorbed Iron in Patients with Low Plasma Latent Iron-Binding Capacity

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