A Quantitative Method for Measuring the Gastrointestinal Absorption of Iron

By John D. Bonnet, Albert B. Hagedorn and Charles A. Owen, Jr.

The availability of radioisotopes of iron has made it possible to quantitate the metabolic cycle of iron with respect to the erythrocyte. With previous technics using radioiron, however, the fraction of an oral dose of iron absorbed by normal persons is relatively small—2 to 27 per cent—which limits the interpretation of results in studying the gastrointestinal absorption of iron. Particularly, these technics are inadequate for use in syndromes of decreased iron absorption.

It seems that the ideal test for absorption of iron would have normal values approaching 50 per cent of the oral dose, so that both increased and decreased absorption could be quantitated. The currently proposed test was designed to achieve this. Two factors helped to increase normal absorption to the 50 per cent level: limiting the dose of iron to 50 μg., and accentuating absorption by the addition of ascorbic acid. Because of the sensitivity of the scintillation detector employed, a 1 μc. dose of Fe⁹⁹ was found to be an adequate tag. The method, as outlined herein, is simple, relatively short, and conveniently quantitative.

Materials

Fe⁹⁹ as ferric chloride or ferrous citrate routinely was prepared so that the usual dose of about 1 μc. was contained in 1 ml. of solution prepared for oral use.

Nonradioactive “carrier” iron was supplied in a solution of ferrous ammonium sulfate, or more often a standard solution stabilized with sulfuric acid as described by Ramsay. This was added to the solution of radioiron to make up any desired quantity, the amount of iron in the radioiron solution being considered in the calculation of total stable iron.

The ascorbic acid mentioned was added to the iron solutions in unmeasured scoopfuls approximating 300 mg.

Methods

The radioiron solution was mixed with a solution of stable iron in a wax-coated drinking cup, and then the ascorbic acid was dissolved in it. The subject drank the rinsings as well as the solution. Less than 0.1 per cent of the Fe⁹⁹ was found to adhere to the cup.

Stool collections were begun and continued until less than 1 per cent of the dose was found in any single specimen.

Radioiron was measured directly in the stool carton over a probe counter consisting...
of a number of crystals of sodium iodide (thallium-activated) embedded in silicone, and optically connected to a 5 inch photomultiplier tube. Since the sides of the stool cartons were vertical, the height of the compressed specimen was a function of its volume. Geometric correction for varying heights of solutions or feces containing radioiron was based on a standard calibration curve. One μc. of Fe₅⁹, in a small volume in the stool carton, yielded about 2200 counts per second—meaning that the efficiency was 6 per cent—over a background of about 4 counts per second. The fecal Fe₅⁹ was determined as a percentage of the oral dose by comparing the radioactivity of a stool specimen with a pilot prepared from the same radioiron solution used for the oral dose. The fraction of the dose not excreted was assumed to have been absorbed.

Checks on methods.—Duplicate tests were given to three normal subjects as a check on reproducibility of the method. Fe₅⁹ absorption, expressed as a percentage of the oral dose, was 75 and 61 per cent, 54 and 34 per cent, and 65 and 62 per cent in these three individuals.

Another check on the validity of the fecal excretion method is measurement of Fe₅⁹ that has been absorbed and incorporated into newly formed red cells. To measure the Fe₅⁹ appearing in the circulating erythrocytes, blood was drawn with a heparinized syringe 14 or more days after the oral dose of radioiron had been given. Fe₅⁹ was assayed in a well counter containing a sodium iodide (thallium-activated) crystal measuring 2 inches in diameter. One μc. yielded about 10,000 counts per second (27 per cent efficiency) over a background of about 4 counts per second at the usual attenuator-discriminator settings. Erythrocytic Fe₅⁹ was calculated on the basis of a whole-blood volume of 70 ml. per Kg. of body weight; results were expressed as a percentage of the orally administered dose. In seven studies the sum of the measured circulating (erythrocytic) and fecal Fe₅⁹ ranged from 90 to 110 per cent of the oral dose, with a mean of 102 per cent.

Subjects

Sixty-three women and 42 men, all apparently healthy, volunteered for the control studies. Eleven (seven women, four men) participated in more than one study. Since the usual dose of Fe₅⁹ was 1 μc., the “permissible” dose² of 11 μc. was not approached even with repeated tests.

Six patients (three women, three men) with nontropical sprue, two additional male patients with steatorrhea from other causes (one with regional enteritis, one having undergone gastrectomy for gastric lymphoma), and seven patients (six women, one man) with hypochromic anemia were compared with the normal series.

Preliminary Experiments

Effect of quantity of carrier iron.—To a solution containing 1 μc. of Fe₅⁹ and about 300 mg. of ascorbic acid was added sufficient stable iron to make a total of 1, 10, 50, 100, 150, 300, or 1000 μg. of stable iron in the dose. The iron absorption with these varying doses, as a percentage of the oral dose, is shown in figure 1. Of the larger doses of stable iron, more actual micrograms of iron were absorbed; but in terms of percentage, the absorption of iron from the larger doses was less. For example, 0.62 μg. of a 1 μg. dose of iron was absorbed, representing 62 per cent; and 110 μg. of a 1000 μg. dose was absorbed, representing 11 per cent.

On the basis of these observations, a dose of 50 μg. of stable iron was selected for routine testing, since normal persons absorbed approximately 50 per cent of this dose.

Effect of eating.—Results in three subjects given a 1 μg. dose of iron 2 hours after eating and in five other subjects given an equal dose after a 12 hour fast showed comparable average absorption values: 52 and 61 per cent, respectively. Absorption of oral iron seemed to be significantly reduced in two subjects (9 and 29 per cent absorption) when the dose was given within an hour after a meal.

Effect of ascorbic acid.—The prevailing view is that in man ferrous iron is better absorbed than ferric iron⁴ and that reduction of valence is the mechanism by which ascorbic acid increases absorption of ferric iron. Certainly the effect of the vitamin has
been recognized by several investigators\textsuperscript{3-6}, and the following experiment seemed to confirm the consensus. Four male subjects were given 0.5 \( \mu \)g. doses of ferric iron containing Fe\textsuperscript{59}, and three other men received the same dose plus about 300 mg. of ascorbic acid. In absence of the vitamin, absorption of the iron averaged 28 per cent (range 10 to 50 per cent); with the vitamin added, absorption averaged 65 per cent (range 56 to 73).

However, the 28 per cent absorption of ferrous iron (ferrous ammonium sulfate) without ascorbic acid was no greater than that of the iron in trivalent form (ferric chloride), as determined in the next step. Seven subjects (five women, two men) received 50 \( \mu \)g. doses of ferrous iron containing Fe\textsuperscript{59}. The women absorbed 35 per cent (range 15 to 60) of the dose, the men 14 and 17 per cent.

This observation led us to doubt that the effect of ascorbic acid on absorption of iron was related solely to reduction of the ferric iron. To resolve the problem ferrous iron was administered with and without ascorbic acid. Seven normal female subjects received nine doses of ferrous citrate (0.8 \( \mu \)g.) containing Fe\textsuperscript{59}, six with about 300 mg. of ascorbic acid added and three without the vitamin. The absorption averaged 40 per cent (range 21 to 54) for the former and 16 per cent (range 10 to 21) for the latter. In two subjects who received both doses, absorption increased from 10 to 21 per cent and from 18 to 36 per cent when the ascorbic acid was added. As all the iron in this experiment was in the ferrous form, the ascorbic acid appears to influence the absorptive process directly. The doubling of absorption with addition of the vitamin must be attributed to some phenomenon other than its reducing power—for instance, chelation.
METHOD FOR MEASURING GASTROINTESTINAL IRON ABSORPTION

THE “STANDARD TEST”

On the basis of the above observations we designed our “standard test.” At least 2 hours after eating, the subject drank a solution containing 50 μg of stable iron, about 1 μc. of Fe⁵⁹, and about 300 mg of ascorbic acid. The total Fe⁵⁹ recovered in the feces was determined. The difference between the amount given and the amount recovered, expressed as a percentage of the oral dose, was the Fe⁵⁹ absorption figure.

STANDARD RESULTS IN NORMAL SUBJECTS

This technic was applied in 19 normal women and 22 normal men. The women absorbed 62 per cent of the dose (range 36 to 75), the men 40 per cent (range 14 to 62). The distribution of the values is shown in figure 2. The difference between the mean values (22 per cent) is highly significant statistically, since it is more than four times the standard deviation of 4.0 per cent.

The women averaged 28.4 years (21 to 60) and the men 30.2 years (25 to 35). Although absorption seems to diminish with age (table 1), this factor appears inadequate to explain the sex difference found in our normal series of predominantly young persons.

The mean concentration of hemoglobin in the blood of the men was 14.1 Gm. per 100 ml. (range 13.2 to 15.6), and in women it was 12.2 Gm. per 100

**Fig. 2.—Distribution of absorption of Fe⁵⁹-labeled iron in 19 normal female and 22 normal male subjects.**
TABLE 1.—Gastrointestinal Absorption in Normal Subjects of 50 μg. Iron Labeled With Fe59

<table>
<thead>
<tr>
<th>Age (yr.)</th>
<th>No.</th>
<th>Normal males</th>
<th>Mean absorption*</th>
<th>No.</th>
<th>Normal females</th>
<th>Mean absorption*</th>
</tr>
</thead>
<tbody>
<tr>
<td>21–24</td>
<td>6</td>
<td>46.2</td>
<td></td>
<td>3</td>
<td>61.0</td>
<td></td>
</tr>
<tr>
<td>25–29</td>
<td>15</td>
<td>37.9</td>
<td></td>
<td>1</td>
<td>54.0</td>
<td></td>
</tr>
<tr>
<td>&gt;34</td>
<td>1</td>
<td>36.0</td>
<td></td>
<td>4</td>
<td>53.8</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
<td>40.0 ± 2.8f</td>
<td></td>
<td>19</td>
<td>62.0 ± 2.8f</td>
<td></td>
</tr>
</tbody>
</table>

*Expressed as percentage of oral dose.
†One standard deviation.

ml. (range 11.6 to 13.0). Since the highest concentration of hemoglobin among the women was less than the lowest among the men, one might suspect that the hemoglobin concentration in some way controlled the absorption of iron. Such a relationship appears unlikely, however, because no parallel between concentration of hemoglobin and absorption of iron could be demonstrated within normal individuals of either sex. It seems that a true sexual difference characterized each factor separately.

RESULTS IN DISEASE STATES

Steatorrhea.—The standard test was carried out in five men and three women with steatorrhea (table 2). In comparison with the data from the normal subjects, the absorption of radioiron in all but two of these patients was low normal or subnormal. In the group with steatorrhea, no obvious relationship of iron absorption with age, with concentration of hemoglobin in the blood, with iron in the serum, or with stool fats was evident.

Hypochromic anemia.—Significantly increased absorption was found in three of six women with hypochromic anemia (table 2)—an association observed repeatedly by others.6 In two of the six, absorption apparently was decreased below normal. The only male patient with hypochromic anemia, a man 70 years old, absorbed less iron than normal younger men.

COMMENT

Review of available methods.—To measure gastrointestinal absorption of iron, three fundamental technics are available. The oldest, that of a total iron balance, is difficult and time-consuming. The second, a "serum iron tolerance test"10 depends on the degree of elevation of the serum iron level following an oral dose of an iron salt. The assumption that the rise in serum iron is directly proportional to the amount of iron absorbed has been questioned.6

The third technic was pioneered by Hahn, who employed radioiron to trace the stable iron. His group11 quantitated iron absorption by the fraction of the dose eventually appearing in circulating erythrocytes. Dubach and associates7 found that the radioiron incorporated in erythrocytes was not valid as an index of absorption in all clinical conditions. Bothwell and associates12 modified Hahn's technic so as to avoid this criticism. Two radioisotopes of iron were given to the subject, one orally (Fe55) and the other
METHOD FOR MEASURING GASTROINTESTINAL IRON ABSORPTION

TABLE 2.—Gastrointestinal Absorption in Patients with Steatorrhea and with Hypochromic Anemia of 50 μg. Iron Labeled With Fe\(^{59}\)

<table>
<thead>
<tr>
<th>Age, sex</th>
<th>Hgb. (Gm./100 ml. blood)</th>
<th>Serum Fe (μg./100 ml.)</th>
<th>Stool fat (% of solids)</th>
<th>Fe(^{59}) absorption (% oral dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steatorrhea</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>46M*</td>
<td>10.0</td>
<td>23</td>
<td>32</td>
<td>6</td>
</tr>
<tr>
<td>48M</td>
<td>10.3</td>
<td>25</td>
<td>58</td>
<td>17</td>
</tr>
<tr>
<td>43M†</td>
<td>10.0</td>
<td>77</td>
<td>33</td>
<td>18</td>
</tr>
<tr>
<td>56M</td>
<td>7.0</td>
<td>25</td>
<td>47</td>
<td>32</td>
</tr>
<tr>
<td>31M</td>
<td>14.2</td>
<td>57</td>
<td>57</td>
<td>54</td>
</tr>
<tr>
<td>56F</td>
<td>12.9</td>
<td>30</td>
<td>63</td>
<td>11</td>
</tr>
<tr>
<td>40F</td>
<td>13.3</td>
<td>140</td>
<td>55</td>
<td>26</td>
</tr>
<tr>
<td>62F</td>
<td>11.9</td>
<td>80</td>
<td>59</td>
<td>37</td>
</tr>
<tr>
<td>Hypochromic Anemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>70M</td>
<td>9.5</td>
<td>33</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>21F</td>
<td>9.7</td>
<td>27</td>
<td>97</td>
<td></td>
</tr>
<tr>
<td>36F</td>
<td>9.7</td>
<td>48</td>
<td>96</td>
<td></td>
</tr>
<tr>
<td>57F</td>
<td>7.0</td>
<td>475†</td>
<td>91</td>
<td></td>
</tr>
<tr>
<td>41F</td>
<td>10.4</td>
<td>38</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>50F</td>
<td>6.5</td>
<td>26</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>66F</td>
<td>8.5</td>
<td>15</td>
<td>17</td>
<td></td>
</tr>
</tbody>
</table>

*Regional ileitis; had had multiple resections.
†Postgastrectomy.
‡After cortisone therapy, 25 mg. three times daily.
§After parenteral medication with iron.

intravenously (Fe\(^{59}\)). Absorption of the oral iron could be quantitated by the amount appearing in the erythrocytes after applying whatever correction was indicated by the efficiency of utilization of the parenteral radioiron.

The method most commonly used today simply determines the difference between the amount of radioiron given orally and that recovered in the feces, as suggested by Moore and Dubach. Results with various radioiron technics are summarized in table 3. As can be seen, the values reported usually are less than 20 per cent of the dose; decreased absorption must be assessed statistically.

Significance of results from this test.—The principal disadvantage of the test is a theoretical one—that it is unphysiologic. In a broad sense all methods that attempt to evaluate absorbability of food iron by the administration of test doses of inorganic iron are unphysiologic. The validity of this test, or of any other procedure employing inorganic iron, must depend on actual results with patients. In the preliminary results reported here from cases of steatorrhea, absorption was decreased or normal, in agreement with the findings of Badenoch and Callender. The majority of our small series of patients with hypochromic anemia exhibited increased absorption of iron. These results are sufficiently encouraging to warrant an extension of the current studies.

The present report, however, is concerned primarily with technical aspects of results from a 50 μg. test dose of iron in normal persons.
Table 3.—Summary of Reported Values for Gastrointestinal Absorption of Iron, Using Radioster.

<table>
<thead>
<tr>
<th>Source</th>
<th>Stable iron</th>
<th>Ascorbic acid added (mg.)</th>
<th>No. of subjects</th>
<th>Tests</th>
<th>Iron absorption (per cent of dose) based on:</th>
<th>Fecal Fe&lt;sup&gt;59&lt;/sup&gt;</th>
<th>Erythrocite Fe&lt;sup&gt;59&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dubach et al. 1948</td>
<td>1/Kg.</td>
<td>FeCl&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Yes</td>
<td>8</td>
<td>10</td>
<td>14.6</td>
<td>7.2</td>
</tr>
<tr>
<td>Bothwell et al. 1953</td>
<td>0.02-0.06</td>
<td>FeCl&lt;sub&gt;2&lt;/sub&gt;</td>
<td>40</td>
<td>4F, 4M*</td>
<td>6</td>
<td>18</td>
<td>3.4</td>
</tr>
<tr>
<td>Peterson and Ettinger</td>
<td>1953</td>
<td>FeCl&lt;sub&gt;2&lt;/sub&gt;</td>
<td>None</td>
<td>4</td>
<td>6</td>
<td>2.7</td>
<td>2.1</td>
</tr>
<tr>
<td>Badenoch and Callender</td>
<td>1956</td>
<td>FeSO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>None</td>
<td>2F, 1M*</td>
<td>5</td>
<td>6.5</td>
<td>8.0</td>
</tr>
<tr>
<td>Chodos et al. 1957</td>
<td>4-80</td>
<td>FeSO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>300-1000</td>
<td>27</td>
<td>19.8</td>
<td>4-33</td>
<td>1-31</td>
</tr>
<tr>
<td>Smith and Mallett 1957</td>
<td>5</td>
<td>FeCl&lt;sub&gt;2&lt;/sub&gt;</td>
<td>50</td>
<td>10</td>
<td>15</td>
<td>27 (8-42)†</td>
<td>19.8</td>
</tr>
<tr>
<td>Bothwell et al. 1958</td>
<td>1/Kg.</td>
<td>FeSO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>100-1000</td>
<td>4</td>
<td>14.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2/Kg.</td>
<td>FeSO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>100-1000</td>
<td>5</td>
<td>6.4†</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*F = female, M = male.
†Range.
‡10.6 per cent if ascorbic acid omitted.
§6.4 per cent if ascorbic acid omitted.

Summary

A simple test is proposed for evaluating the gastrointestinal absorption of iron. It begins with an oral dose of 50 μg. of stable iron labeled with 1 μc. of Fe<sup>59</sup>, to which about 300 mg. of ascorbic acid are added. Feces are assayed for Fe<sup>59</sup> until any single stool contains less than 1 per cent of the dose. Absorption is determined on the assumption that all unrecovered Fe<sup>59</sup> has been absorbed. The test was designed to minimize handling of stools by assaying whole specimens, and this process has the added virtue of increasing statistical accuracy of the measurement of radioactivity.

The main advantage of the procedure using a 50 μg. dose is that with it one obtains a normal value for absorption which is about one-half of the dose. This permits measurement of either increased or decreased iron absorption.

With our standard test method, 19 normal women absorbed an average of 62 per cent of the dose and 22 normal men absorbed 40 per cent. In addition to this sex difference, not explained by the difference in their blood hemoglobin levels, there seemed to be a tendency toward reduced absorption with greater age.

Preliminary results of this test in patients with hypochromic anemia and steatorrhea are reported.

Increased absorption of iron followed the administration of ascorbic acid whether the iron was in the ferric or the ferrous form. The concept that ferrous iron is better absorbed by man requires re-evaluation.

Summario in Interlingua

Es proponite un simple test pro evalutar le absorption gastrointestinal de ferro. Illo comencia con un dose oral de 50 μg de ferro stabile marcate con 1 μc de Fe<sup>59</sup> a que es addite circa 300 mg de acido ascorbic. Le feces es studiate pro Fe<sup>59</sup> usque un evacuation individual contine minus que 1 pro cento del dose.
METHOD FOR MEASURING GASTROINTESTINAL IRON ABSORPTION

Le absorption es calculate con le premissa que omne le non-excernite Fe50 es absorbite. Le test esseva disveloppate pro reducer le manipulation de feces. Isto es realisate in le presente methodo in tanto que illo require le essayage de specimen total. Illo ha le virtute additional que illo augmenta le accuratia statistic del mesuration de radioactivitate.

Le major avantage de usar un dose de 50 µg es que on obtene assi un valor normal pro le absorption que es approximativemente un medietate del dose. Isto permitte le mesuration de augmentos si ben como reductiones del absorption de ferro.

Con le uso de nostre methodo standard pro le test, nos ha constatate que 19 normal femininas absorbeva al media 62 pro cento del dose, durante que 22 normal homines absorbeva 40 pro cento. A parte iste differentia inter le sexos (que non es explicate per un differentia in le nivellos de hemoglobina), il pareva exister un tendentia verso un reduction de absorption con le avantiamento del etate.

Es reportate resultatos preliminari de iste test in patientes con anemia hypochromic e steatorrhea.

Augmento del absorption de ferro sequeva le administration de acido ascorbic, sin reguardo a si le ferro esseva in forma ferric o ferrose. Le conception que ferro ferrose es melio absorbite per le organismo human require un reevaluation.

REFERENCES


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