The Influence of Amino Acids on Iron Absorption

By Donald Kroe, Thomas D. Kinney, Nathan Kaufman and J. V. Klavins

Previous experiments have demonstrated that the composition of the diet influences the amount of iron absorbed from the gastrointestinal tract. It has been shown that diets low in protein are associated with a low iron uptake and that the amount of iron absorbed is roughly dependent upon the amount of protein in the diet.\(^1\)\(^2\) Further, it was found that it is necessary for a diet to contain from 15–18 per cent protein for adequate iron absorption.\(^1\)\(^2\) The manner in which protein exerts this effect is not known, but it has been suggested that one of the factors enhancing iron absorption could be the availability of increased amounts of amino acids derived from high dietary protein.\(^2\) Since it has been shown that amino acids have a chelating effect upon iron,\(^3\)\(^5\) the possibility was considered that the increase in absorption of iron with increased protein might be related to this phenomenon to some degree. For this reason, a series of experiments was devised to determine the relative effect of a number of amino acids upon iron absorption from the gastrointestinal tract.

Materials and Methods

Nine amino acids, as well as phosphate buffer alone, were tested on groups of rats. The amino acids were: 1-methionine, 1-proline, 1-phenylalanine, 1-serine, 1-glutamic acid, 1-asparagine, 1-histidine, 1-ethionine, and 1-glutamine. They were selected in order to give a wide range of structural types. Male albino rats of the Holtzman strain, weighing approximately 125 Gm. each, and with hemoglobin values of 13.5 ± 0.7 Gm. per cent were used. For each amino studied, seven rats were used, except for phenylalanine, when six rats were used. Iron in the form of Fe\(^{59}\)SO\(_4\) with a specific activity of 25.1 mc./mg., was used in all experiments. Test solutions were prepared by adding an amino acid and Fe\(^{59}\)SO\(_4\) to aliquots of 0.1 M phosphate buffer so that the resulting Fe\(^{59}\) activity was 20 sc./ml., the amino acid concentration was 0.1 M, and pH ranged from 3.2–3.4. A control solution was prepared by adding Fe\(^{59}\)SO\(_4\) to an aliquot of the stock phosphate buffer with a resulting pH of 3.3. The composition of the control solution was the same as the test solutions but without an amino acid.

The rats were fasted for 24 hours in order to minimize any variable dietary influences on iron absorption. They were then anesthetized with sodium pentobarbital. The abdomen was opened, the small intestine was ligated at the pylorus, and at a point 4 cm. distal to the pylorus. Proximal to the second ligature, a third was prepared but not tied. At this third point an incision was made through the bowel wall and the intestinal lumen thus exposed was rinsed with 2–3 ml. of Ringer’s solution. A needle, whose tip was covered with a length of plastic tubing, was inserted into the lumen. The third ligature was secured around the needle, 0.4 ml. of test or control solution was delivered into the lumen, the needle removed, and the third ligature tied. The abdominal incision was then sutured.

At time periods of 5, 10, 15, 30, 45 and 60 minutes after the administration of the solution into the intestinal lumen, 20 mm.\(^3\) blood samples were collected from the tail.
The mean activity of Fe<sup>59</sup> in the blood after introduction of iron into the duodenum is illustrated in figure 1 and table 1. There was greater activity when the iron was administered with an amino acid than when it was administered with buffer alone. There was a statistically significant difference in the mean blood Fe<sup>59</sup> activity between the rats receiving buffer and those receiving any of the amino acids studied, with the exception of histidine and ethionine at the 5-minute interval. The p values were > 0.10 for histidine and > 0.05 for ethionine at 5 minutes. Significant differences were at the 5 per cent level for methionine at 5 minutes, for histidine, proline, and phenylalanine at 15 minutes, for phenylalanine and serine at 30 minutes and for serine at 60 minutes. All the other determinations were significant at the 1 per cent level.

The effect of the amino acids on the rate of absorption of iron was determined by taking the difference between adjacent time points and dividing
Table 1.—Mean Activity of Fe\(^{59}\) in Blood (Count per 5 Minutes per 20 mm.\(^3\)) When Various Amino Acids and Phosphate Buffer Were Administered

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>5 min</th>
<th>10 min</th>
<th>15 min</th>
<th>30 min</th>
<th>45 min</th>
<th>60 min</th>
<th>Number of Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histidine</td>
<td>91</td>
<td>229</td>
<td>386</td>
<td>762</td>
<td>1012</td>
<td>1163</td>
<td>7</td>
</tr>
<tr>
<td>Asparagine</td>
<td>281</td>
<td>600</td>
<td>823</td>
<td>946</td>
<td>1057</td>
<td>1029</td>
<td>7</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>179</td>
<td>486</td>
<td>819</td>
<td>1047</td>
<td>1112</td>
<td>1100</td>
<td>7</td>
</tr>
<tr>
<td>Glutamine</td>
<td>227</td>
<td>651</td>
<td>883</td>
<td>1103</td>
<td>1139</td>
<td>1123</td>
<td>7</td>
</tr>
<tr>
<td>Ethionine</td>
<td>134</td>
<td>424</td>
<td>579</td>
<td>718</td>
<td>727</td>
<td>697</td>
<td>7</td>
</tr>
<tr>
<td>Methionine</td>
<td>171</td>
<td>417</td>
<td>510</td>
<td>642</td>
<td>648</td>
<td>636</td>
<td>7</td>
</tr>
<tr>
<td>Proline</td>
<td>182</td>
<td>355</td>
<td>458</td>
<td>608</td>
<td>660</td>
<td>662</td>
<td>7</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>178</td>
<td>345</td>
<td>445</td>
<td>506</td>
<td>543</td>
<td>545</td>
<td>6</td>
</tr>
<tr>
<td>Serine</td>
<td>225</td>
<td>448</td>
<td>515</td>
<td>525</td>
<td>540</td>
<td>510</td>
<td>7</td>
</tr>
<tr>
<td>Phosphate buffer</td>
<td>63</td>
<td>117</td>
<td>145</td>
<td>184</td>
<td>201</td>
<td>194</td>
<td>7</td>
</tr>
<tr>
<td>Pooled standard</td>
<td>78</td>
<td>58</td>
<td>223</td>
<td>252</td>
<td>240</td>
<td>231</td>
<td></td>
</tr>
</tbody>
</table>

Note: To obtain "P" values, 59 degrees of freedom were used where

\[
t = \frac{\text{Difference of means}}{\text{pooled S.D.} \times \sqrt{2/N}},
\]

by the number of intervening minutes. The mean rates of appearance of Fe\(^{59}\) in blood are shown in table 2.

In the 0–5 minute interval, the rate of appearance of Fe\(^{59}\) in the blood was significantly higher in the rats treated with amino acid when compared with phosphate buffer, except for histidine and ethionine. Neither was significant at the 5 per cent level, while all the others were significant at the 1 per cent level.

At the 5–10 minute interval, histidine was the only amino acid which was not associated with a significant difference in rate of appearance of Fe\(^{59}\) in the blood. The p values for proline and phenylalanine were < 0.05, whereas in all the others it was < 0.01.

At the 10–15 minute interval, serine, phenylalanine, proline and methionine were not associated with a significant difference when compared with the buffer; all others showed a significant difference at the 1 per cent level.

In the 15–30 minute interval, the only statistically significant differences were with proline at the 5 per cent level and glutamine, glutamic acid and histidine at the 1 per cent level.

During the last two intervals there was no statistically significant difference in the rate of appearance of the Fe\(^{59}\) except for the rats treated with histidine and this was significant at the 1 per cent level.

At the end of the experiment—that is, 60 minutes after the introduction of Fe\(^{59}\) into the duodenum—there was significantly less iron in the livers of animals receiving phosphate buffer than in the livers of any of the animals receiving Fe\(^{59}\) together with an amino acid (table 3). At the end of the experiment the pH of the contents of the intestinal loop ranged from 7.2–8.9.
Table 2.—Rate of Appearance of Fe$^{59}$ in Blood

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>0-5</th>
<th>5-10</th>
<th>10-15</th>
<th>15-30</th>
<th>30-45</th>
<th>45-60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histidine</td>
<td>18.3</td>
<td>27.5</td>
<td>31.4</td>
<td>25.0</td>
<td>16.6</td>
<td>10.1</td>
</tr>
<tr>
<td>Asparagine</td>
<td>56.2</td>
<td>63.8</td>
<td>44.5</td>
<td>8.2</td>
<td>7.4</td>
<td>-1.8</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>35.8</td>
<td>61.4</td>
<td>66.6</td>
<td>15.2</td>
<td>4.3</td>
<td>-0.8</td>
</tr>
<tr>
<td>Glutamine</td>
<td>45.3</td>
<td>84.8</td>
<td>46.4</td>
<td>14.7</td>
<td>2.4</td>
<td>-1.1</td>
</tr>
<tr>
<td>Ethionine</td>
<td>28.8</td>
<td>58.1</td>
<td>31.0</td>
<td>9.2</td>
<td>0.6</td>
<td>-2.0</td>
</tr>
<tr>
<td>Methionine</td>
<td>34.3</td>
<td>49.2</td>
<td>18.4</td>
<td>8.8</td>
<td>0.4</td>
<td>-0.8</td>
</tr>
<tr>
<td>Proline</td>
<td>36.5</td>
<td>34.5</td>
<td>20.6</td>
<td>10.0</td>
<td>3.5</td>
<td>0.1</td>
</tr>
<tr>
<td>Phenylnalnine</td>
<td>35.6</td>
<td>21.1</td>
<td>20.1</td>
<td>4.1</td>
<td>2.5</td>
<td>0.1</td>
</tr>
<tr>
<td>Serine</td>
<td>45.0</td>
<td>44.6</td>
<td>13.4</td>
<td>0.8</td>
<td>1.0</td>
<td>-2.0</td>
</tr>
<tr>
<td>Phosphate buffer</td>
<td>12.5</td>
<td>10.8</td>
<td>5.6</td>
<td>2.6</td>
<td>1.1</td>
<td>-0.5</td>
</tr>
<tr>
<td>Pooled standard</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>error of mean</td>
<td>6.0</td>
<td>8.7</td>
<td>5.7</td>
<td>2.5</td>
<td>2.7</td>
<td>1.9</td>
</tr>
<tr>
<td>Standard error of</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>difference in two</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean rates</td>
<td>8.4</td>
<td>12.2</td>
<td>8.1</td>
<td>3.6</td>
<td>3.8</td>
<td>2.7</td>
</tr>
</tbody>
</table>

**Note 1:** Both standard errors are computed for n = 7 and are used only for making comparisons within a time period. To obtain "P" values, 59 degrees of freedom were used where

\[
t = \frac{\text{Difference of means}}{\text{Standard error of difference in two mean ratios}}.
\]

**Note 2:** To compare results in two time periods, the covariances must be taken into account. Then the proper standard error used is

\[
\sqrt{(s_{11} - 2s_{1j} + s_{jj})/7}, \text{ where } s_{11} = \text{variance at time } i, \\
\quad s_{jj} = \text{variance at time } j, \\
\quad s_{ij} = \text{covariance of time } i \text{ with time } j.
\]

**Note 3:** Table of the variances and covariances:

<table>
<thead>
<tr>
<th>i or j =</th>
<th>i or j =</th>
<th>i or j =</th>
<th>i or j =</th>
<th>i or j =</th>
<th>i or j =</th>
<th>i or j =</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>248</td>
<td>275</td>
<td>73</td>
<td>-1</td>
<td>-45</td>
<td>-22</td>
</tr>
<tr>
<td>2</td>
<td>523</td>
<td>144</td>
<td>8</td>
<td>-62</td>
<td>-35</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>231</td>
<td>19</td>
<td>-19</td>
<td>-19</td>
<td>-26</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>45</td>
<td>5</td>
<td>-14</td>
<td>49</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>49</td>
<td>19</td>
<td>26</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**DISCUSSION**

It is evident from these experiments that iron appears more quickly and in greater amounts in the blood if it is administered with an amino acid than with phosphate buffer alone. The amount of circulating iron is a reflection of three factors: (1) the amount and rate of absorption, (2) the amount and rate of deposition in the tissues, and (3) the release of iron from the tissues into the circulation. The fact that the liver iron content at the end of the experiment was significantly higher in the animals receiving
Table 3.—Fe<sup>59</sup> Activity in Liver after 60 Minutes

<table>
<thead>
<tr>
<th>Substance Administered with Fe&lt;sup&gt;59&lt;/sup&gt;</th>
<th>Counts per 5 Minutes per Gm. on Each of 2 Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamine</td>
<td>18,916, 17,222</td>
</tr>
<tr>
<td>Proline</td>
<td>10,846, 12,298</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>16,726, 15,511</td>
</tr>
<tr>
<td>Ethionine</td>
<td>11,107, 14,713</td>
</tr>
<tr>
<td>Methionine</td>
<td>16,976, 15,882</td>
</tr>
<tr>
<td>Histidine</td>
<td>19,420, 16,907</td>
</tr>
<tr>
<td>Serine</td>
<td>11,089, 13,438</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>19,873, 20,327</td>
</tr>
<tr>
<td>Asparagine</td>
<td>17,906, 20,481</td>
</tr>
<tr>
<td>Phosphate buffer</td>
<td>4,398, 3,455</td>
</tr>
</tbody>
</table>

The amino acids indicates that the elevated blood-iron activity in these animals is a reflection of increased absorption from the gastrointestinal tract rather than a reflection of altered deposition.

As can be seen from the curves in figure 1 the rate (table 2) and quantity (table 1) of Fe<sup>59</sup> appearing in blood was not identical for the different amino acids studied.

The amino acids studied could be grouped into three categories on the basis of patterns of the curves obtained for the rate and amount of Fe<sup>59</sup> appearing in the serum. Category I consists of glutamine, glutamic acid, and asparagine which were associated with high blood Fe<sup>59</sup> values; Category II consists of methionine, ethionine, proline, serine and phenylalanine which were associated with lower Fe<sup>59</sup> blood values; Category III consists of histidine which was associated with an entirely different pattern in blood Fe<sup>59</sup> content. The amount and rate of appearance of blood Fe<sup>59</sup> associated with histidine administration were the lowest for all the amino acids studied at the beginning of the experiment but increased so that by the end of the experiment the blood levels were the highest observed.

The amount of blood Fe<sup>59</sup> activity did not correlate with the pH values of the isolated gut segment at the end of the experiment. The pH of the phosphate buffer-Fe<sup>59</sup> solution was 3.3; the pH of the contents of the isolated segment was 8.02 at the end of the experiment 1 hour later. The pH of the solutions containing glutamine was 3.43, that containing glutamic acid was 3.45, while at the end of this experiment the pH of the contents of the segments was 8.45 and 7.57 respectively. The Fe<sup>59</sup> count in the blood was high when these amino acids were used. As mentioned above, the pH of the con-
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tents of the isolated segment of the phosphate buffer Fe50 animal was between
these two, i.e., 8.02, and yet the blood Fe50 count was significantly lower when
compared with the high blood Fe50 counts of the animals given amino acids.
What effect the pH changes may have had during the course of the experiments
was not determined.
Höber, in 1903, suggested that protein breakdown products participated
in the absorption of iron. It has been shown subsequently that amino acids
in general are effective chelating agents5 and that certain of these will affect
the absorption of iron.7 It can be postulated that amino acids facilitate ab-
sorption by chelating iron in the lumen of the gut, acting as vehicles for its
absorption. The results of the present experiment add support to this theory.

SUMMARY

A series of experiments were devised to determine the effect of various
amino acids upon the absorption of iron from the gastrointestinal tract.
This was done by introducing single amino acids and Fe50 into isolated loops
of small intestine and measuring the serum for radioactivity and the uptake
of Fe50 by the liver. It was found that under the conditions of the experiments
all of the amino acids studied effected an increase in the serum iron and iron
deposition in the liver.

SUMMARIO IN INTERLINGUA

Esseva elaborate un serie de experimentos pro determinar le effecto de
varie amino-acidos super le absorption de ferro ab le vias gastrointestinal.
Isto esseva complite per introducer amino-acidos individual insimul con
Fe50 in isolate ansas de intestino tenue e mesurar le radioactivitate del sero e
e le quantitate de Fe50 acceptate per le hepate. Esseva trovate que sub le
conditiones del experimentos omne le amino-acidos studiate induciva un
augmento del ferro del sero e del deposition de ferro in le hepate.

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