Iron Absorption: Effects of Sugars and Reducing Agents

By SIMEON POLLACK, RICHARD M. KAUFMAN AND WILLIAM H. CROSBY

ALTHOUGH many substances have been shown to increase iron absorption,1,2 the mechanism of this action is obscure. Fructose, for example, increases iron absorption in rats, and it has been suggested that its effect depends on its ability to chelate iron.4 We proposed to test this hypothesis, and to investigate possible relationships between the effects of various metabolic substrates and reducing agents on iron absorption.

METHOD

Iron absorption was studied in WRCF rats* (a derivative of the Wistar strain) weighing 200–350 Gms., utilizing repeated total body counting to estimate iron50 absorption.6 The animals, fasted overnight, were lightly anesthetized with ether and dosed through an esophageal feeding needle with 1 ml. of the solutions to be tested. Each ml. of control solution contained 250 μg. of iron as ferric chloride, tracer amounts of iron50 chloride, and 1.6 mg. of ascorbic acid; the molar excess of ascorbic acid assured total reduction of ferric ion to ferrous ion. Experimental solutions were identical with the control solution, except for the addition of 0.4 mM of the substance being tested except where otherwise noted. pH of the control solution was 2.4. When the test material in the experimental solution changed the pH (always more acid), the control solution for that experiment was brought to the same pH with HCl. Materials tested included fructose, glucose, galactose, pyruvate, lactate, cysteine and hydroquinone.

RESULTS AND DISCUSSION

Fructose has been shown to increase iron absorption in rats.4 In our experiments the increase occurred consistently but was variable in magnitude (table 1). The effect of fructose on iron absorption has been attributed to its ability to form a stable complex with iron, thereby facilitating the transport of iron across the intestinal mucosa. Fructose is not unique in its ability to combine with iron. Glucose and galactose also combine with iron,7 but have no effect on iron absorption (table 2).

Fructose, unlike glucose and galactose, enters into the metabolism of the rat's intestinal tissues to a variable extent while being absorbed in vivo. It appears in the rat's portal blood as a mixture of fructose, glucose and lactate, the lactate comprising from 2 per cent to 60 per cent of the absorbed fructose.8 Glucose and galactose are absorbed in vivo or in perfused rat gut with little or no participation in mucosal metabolism, appearing largely unchanged in the portal blood.9,10

Pyruvate and lactate, the final products of glycolysis, increase iron absorp-
Table 1.—The Effect of Fructose on Iron\(^{59}\) Absorption

<table>
<thead>
<tr>
<th>Group</th>
<th>No. Rats</th>
<th>% Fe(^{59}) Absorption Mean ± SD</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>10.1 ± 3.7</td>
<td></td>
</tr>
<tr>
<td>Experimental</td>
<td>10</td>
<td>15.7 ± 5.7</td>
<td>&lt;0.05†</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>18.5 ± 7.9</td>
<td></td>
</tr>
<tr>
<td>Experimental</td>
<td>10</td>
<td>19.6 ± 5.8</td>
<td>N.S.†</td>
</tr>
</tbody>
</table>

*Statistical comparison is made between control and experimental group using the standard two tailed "t" test.
N.S. = not significant at the .05 level.
†These two experiments are representative. Iron absorption was consistently increased in groups receiving fructose in 4 separate experiments. The increase was statistically significant in two.

Table 2.—The Effect of Glucose and Galactose on Iron\(^{59}\) Absorption

<table>
<thead>
<tr>
<th>Group</th>
<th>No. Rats</th>
<th>% Fe(^{59}) Absorption Mean ± SD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>20</td>
<td>14.2 ± 4.6</td>
<td></td>
</tr>
<tr>
<td>Experimental</td>
<td>20</td>
<td>12.2 ± 5.2</td>
<td>N.S.</td>
</tr>
<tr>
<td>Galactose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>20</td>
<td>14.9 ± 7.2</td>
<td></td>
</tr>
<tr>
<td>Experimental</td>
<td>20</td>
<td>15.8 ± 5.8</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

ATP and DPNH are both generated during metabolism. Both have been shown to be important in biological iron transfers.\(^{11,12,13}\) It has been suggested that iron in a state of transfer from proteins or through membranes must be in the reduced form.\(^{11}\) It is conceivable that the metabolism of fructose, lactate, or pyruvate affects iron absorption by generating DPNH, the DPNH facilitating iron absorption by reducing intramucosal ferric ion, or preserving the reduced state of ferrous iron.\(^{14}\) If lactate affected iron absorption by virtue of generating DPNH, and if DPNH acted as an intracellular reducing agent, then we anticipated that a maximally effective dose of a reducing agent might mask the effect of simultaneously administered lactate. However, when hydroquinone was used as the reducing agent, in a dose adequate to insure its maximum effect in increasing iron absorption,\(^{14}\) lactate further increased iron absorption (table 4), suggesting that hydroquinone and lactate act independently on iron absorption. If the effect of both is on the valence state of intramucosal iron then the sites of their actions must be at separate loci in the transport system. The concept of separate loci of action of reducing substances in iron absorption is rendered plausible (but by no means proved) by the effect of cysteine, a strong reducing agent, in increasing iron absorption in the presence of a maximally effective dose of hydroquinone (table 5).
Table 3.—The Effect of Lactate and Pyruvate on Iron Absorption

<table>
<thead>
<tr>
<th>Group</th>
<th>No. Rats</th>
<th>% Fe Absorption</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>20</td>
<td>10.6 ± 6.3</td>
<td></td>
</tr>
<tr>
<td>Experimental</td>
<td>20</td>
<td>14.3 ± 7.4</td>
<td>.05 &lt; p &lt; .1</td>
</tr>
<tr>
<td>Pyruvate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>20</td>
<td>11.7 ± 5.2</td>
<td></td>
</tr>
<tr>
<td>Experimental</td>
<td>20</td>
<td>16.2 ± 5.6</td>
<td>.01</td>
</tr>
</tbody>
</table>

*Lactic acid was used to make the experimental solution. The pH of the experimental and control solutions was 1.8.
†Sodium pyruvate was used to make the experimental solution. The pH of the experimental and control solutions was 1.8.

Table 4.—The Effect of Hydroquinone with and without Lactate on Iron Absorption

<table>
<thead>
<tr>
<th></th>
<th>No. Rats</th>
<th>% Fe Absorption</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroquinone</td>
<td>10</td>
<td>20.2 ± 7.7</td>
<td></td>
</tr>
<tr>
<td>Hydroquinone + Lactate</td>
<td>10</td>
<td>35.0 ± 7.0</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

*Each rat was given 35 mg. of hydroquinone. Those rats given lactate received 0.8 mM. The pH of the control and experimental solutions was 1.7.

Table 5.—The Effect of Hydroquinone with and without Cysteine on Iron Absorption

<table>
<thead>
<tr>
<th></th>
<th>No. Rats</th>
<th>% Fe Absorption</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroquinone</td>
<td>10</td>
<td>24.5 ± 10.5</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>Hydroquinone + Cysteine</td>
<td>11</td>
<td>34.3 ± 6.0</td>
<td></td>
</tr>
</tbody>
</table>

*35 mg. of hydroquinone was given in each dose. pH of control and experimental solutions was 1.1.

In vitro experiments have shown that iron absorption by gut sacs is dependent on cellular metabolism. The increase in iron absorption produced by fructose, lactate and pyruvate in vivo is consistent with these findings. The variable effect of fructose reflects, perhaps, the variable extent to which it is metabolized by the mucosa during its absorption.

Several speculations may be entertained as unifying explanations of these observations: 1) ATP or DPNH, or both, may be involved in changing the rate of iron absorption in vivo; this would imply that the quantity of these compounds in the gut mucosa limits the rate of iron absorption in vivo. This is difficult to accept since the cells are bathed constantly in an abundance of metabolic substrate in the extracellular fluid in vivo. If fructose-generated ATP or DPNH were responsible for increasing iron absorption it would seem likely that the locus of ATP or DPNH generation must bear some special relation to the locus (or loci) of the iron transport system within the mucosal cell. 2) Lactate or pyruvate per se could be responsible for increasing iron absorption. While fructose does not seem to affect iron absorption by virtue of its chelating properties, it does generate lactate and probably pyruvate.
within the mucosa. Pyruvate can itself combine with iron (as evidenced by the generation of a red complex on mixing pyruvate and ferrous chloride in these experiments). Perhaps in so doing it facilitates the transcellular passage of iron during absorption.

The additive effects of cysteine and hydroquinone on iron absorption is consistent with either of these hypotheses. Cysteine may act as a reducing agent at a locus separate from that of hydroquinone, or it may influence iron absorption by functioning as a carrier.

**Summary**

The effect of the iron-chelating sugars, fructose, glucose and galactose, on iron absorption in rats has been examined. Fructose has an effect in increasing iron absorption. Glucose and galactose have no effect on iron absorption. These findings suggest that the metabolism of fructose is responsible for changing iron absorption in the rat since it is metabolized during its absorption, while glucose and galactose are not.

Pyruvate and lactate, the final products of glycolysis, also increase iron absorption in the rat. The effects of lactate and hydroquinone on iron absorption are additive, suggesting independent actions. The effects of cysteine and hydroquinone on iron absorption are additive, suggesting independent actions of these two reducing agents in increasing iron absorption. Alternative hypotheses are offered in explanation of these observations.

**SuMxARIo IN INTERLINGUA**

Esseva examinate le effecto del sucros ferro-chelatori—fructosa, glucosa, e galactosa—super le absorption de ferro in rattos. Fructosa ha le effecto de augmentar le absorption de ferro. Glucosa e galactosa ha nulle effecto super le absorption de ferro. Iste constatationes suggere que le metabolismo de fructosa es responsabile pro alterar le absorption de ferro in rattos, vite que illo es metabolisate durante le processo de su absorption, durante que isto non es le caso pro glucosa e galactosa.

Pyruvato e lactato, le productos final de glycolysis, etiam augmenta le absorption de ferro in le ratto. Le effectos de lactate e de hydroquinona super le absorption de ferro es additive, lo que pare indicar actiones independente. Le effectos de cysteina e hydroquinona es etiam additive, lo que suggere actiones independente etiam pro iste agentes de reduction. Hypotheses alternative in explication del observationes es presentate.

**REFERENCES**

IRON ABSORPTION

deposition in spleen and liver with
5. Jacobi, H., Pfeifer, K., and Rummel, W.: Komplexbildner und aktiver Eisen-
transport durch die Darmwand. Naunyn-Schmeidebergs Archiv. für
body iron in animals using whole-
body liquid scintillation detectors.
7. Charley, P. J., Sarkar, B., Stitt, C., and Saltman, P.: The chelation of iron by
sugars. Biochim. et biophys. acta 69:
313, 1963.
the manner of transport of absorbed
of the C14 compounds recovered in
portal plasma after enteral administra-
tion of C14 glucose. Biochim. et bio-
10. Tzur, R., and Shapiro, B.: Intestinal
absorption of galactose. Biochim. et
11. Lockhead, A. C., and Goldberg, A.: Mechansms in the transfer of protein-
bound iron for haem biosynthesis.
12. Mazur, A., Green, S., and Carleton, A.: Mechanism of plasma iron incorpora-
tion into hepatic ferritin. J. Biol.
13. —, Carleton, A., and Carlsen, A.: Relation of oxidative metabolism to
the incorporation of plasma iron into
ferritin in vivo. J. Biol. Chem. 236:
1109, 1960.
H., and Butkiewicz, J. E.: Reducing
agents and absorption of iron. Nature
15. Dowdle, E. B., Schachter, D., and
Schenker, H.: Active transport of
Fe59 by everted segments of rat duo-

Captain Simeon Pollack, MC, Department of Hematology,
Walter Reed Army Institute of Research, Washington, D. C.

Captain Richard M. Kaufman, MC, Department of Hemato-
logy, Walter Reed Army Institute of Research, Washington,
D. C.

Colonel William H. Crosby, MC, Director, Division of Medi-
cine and Chief, Department of Hematology, Walter Reed
Army Institute of Research, Washington, D. C.
Iron Absorption: Effects of Sugars and Reducing Agents

SIMEON POLLACK, RICHARD M. KAUFMAN and WILLIAM H. CROSBY

Updated information and services can be found at:
http://www.bloodjournal.org/content/24/5/577.full.html

Articles on similar topics can be found in the following Blood collections

Information about reproducing this article in parts or in its entirety may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at:
http://www.bloodjournal.org/site/subscriptions/index.xhtml