Effect of Experimental Chronic Alcohol Ingestion
and Folic Acid Deficiency on Iron Absorption

By Antonio Celada, Hans Rudolf, and Alfred Donath

The effect of chronic alcohol ingestion and folic acid deficiency on iron absorption in rabbits was studied. Iron absorption was measured in a whole-body counter using \(^{59}\)Fe, and plasma iron turnover (PIT) was simultaneously determined with \(^{55}\)Fe. One group was given a 20% ethanol solution for 2 or 6 mo. There was no significant difference in iron absorption (\(p > 0.50\)), while PIT was reduced (\(p < 0.05\)). A dietary folic acid deficiency was induced in the second group. Iron absorption and PIT were increased (\(p < 0.0001\)). A third group of rabbits was given alcohol for 2 mo and food without folic acid. In this group, iron absorption increased (\(p < 0.0001\)), but the PIT remained normal. Administration of folic acid to the last two groups reduced the iron absorption to normal values. Two other groups were given methotrexate, which increased the iron absorption in proportion to the dose. In both groups, PIT was normal. These data suggest that chronic alcohol ingestion in the absence of folic acid deficiency does not modify iron absorption. Folic acid deficiency increases iron absorption by two probable mechanisms: first, by increasing PIT, which is concomitant with ineffective erythropoiesis; and second, by a probable direct effect on the intestinal mucosal membranes.

In a significant proportion of patients with chronic alcoholism, an increase of iron deposits can be demonstrated using direct or indirect methods,\(^1\)\(^-\)\(^4\) and a good correlation between the amount of these deposits and consumption of alcoholic beverages has been reported.\(^5\)

There are currently two hypotheses to explain iron overload in alcoholic cirrhosis. The first one postulates that the increased iron absorption is secondary to the heavy ingestion of iron contained in wine.\(^6\) The fact that some patients consume beverages containing only trace amounts of iron (whisky, gin, and others)\(^7\)\(^-\)\(^8\) does not support this view. According to the second hypothesis, alcohol increases iron absorption at the luminal level.\(^9\) However, recent studies did not confirm this hypothesis.\(^10\) Acute alcohol ingestion does not enhance inorganic iron absorption, and even decreases heme iron absorption significantly.

Folic acid metabolism is altered during chronic alcohol ingestion.\(^11\) Folic acid deficiency can induce a megaloblastic anemia, which alters iron metabolism by increasing plasma iron turnover (PIT). In this work we studied the effects of folic acid deficiency and chronic alcohol ingestion, either together or separately, using rabbits as an experimental animal.

MATERIALS AND METHODS

Animals

The animals used were 4-kg male albino rabbits, aged 4–5 mo at the beginning of the experiment (Kunath S.A., Aarau, Switzerland). Rabbits were chosen because the gut flora of these animals does not produce folic acid.\(^12\)

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Production of Folic Acid Deficiency

Folic acid deficiency was induced by administration of a diet deficient in folic acid. The folic acid was eliminated from the rabbit food (Lacta S.A., Coppet, Switzerland) by boiling for 3 hr and changing water 3 times, and then drying the food. It is possible that some fat or water-soluble vitamins, as well as some mineral components, may be eliminated during this process. These elements were therefore replaced as follows: a mixture of salts (salt mixture 446 (NRAL) I.C.N., Nutritional Biochemicals Corporation, Cleveland, Ohio), as well as water and fat-soluble vitamins, were added according to the Briggs’ diet. Folic acid, as measured by Herbert’s technique, in 5 samples of rabbit food was 78.24 ± 19.21 μg/kg. After folic acid elimination, the amount of this element in the food is not measurable. This procedure induced a folic acid deficiency within 10 wk, as defined by low serum folate, hypersegmentation of granulocytes, macroovalocytosis of erythrocytes, decreased hemoglobin, hematocrit, and increased mean corpuscular volume.

Chronic Administration of Alcohol

In a series of experiments, drinking water was replaced by a 20% ethanol solution. None of the animals refused to drink this solution, with the average consumption being 65 ml/day.

Experimental Procedure

Animals were placed in metabolic cage, and 2 wk prior to the experiment, all rabbits were given intramuscular vitamin B₁₂ (100 μg; B₁₂ Depot, Siegfried A.G., Zofingen, Switzerland) and iron (50 mg; Imferdex, Fisons, A.G., Zug, Switzerland).

The 57 rabbits composing this study were placed into 7 groups (Table 1). A group of 10 rabbits was used as control. Five of these were fed a normal diet for rabbits (Lacta S.A., Coppet, Switzerland) without antibiotics. The other 5 rabbits were fed with the folic-acid-deficient diet, which had been reconstituted with folic acid (pteroylglutamic acid, 3.0 mg/kg of diet). These two diets were begun 10 wk before iron absorption studies were performed. The second group was given alcohol over a period of 2 (10 animals) or 6 mo (5 animals) before the iron absorption studies. These animals were fed normal food and were also given 1 mg of intramuscular folic acid (Folvite, Lederle Laboratories, Pearl River, N.Y.) every 15 days. Hematologic parameters, iron absorption, and ferrokinetics were done 2 wk after the last folic acid injection. In the third group of 10 rabbits, a folic acid deficiency was induced. Group 4, comprised of 5 animals, was given food depleted of folic acid as well as the alcohol solution for 10 wk before the iron absorption studies. After these studies, groups 3 and 4 were given 15 mg of folic acid intramuscularly, and 2 wk later their iron absorption was retested. Group 5 (5 rabbits) was given a normal diet, however iron absorption was tested after diluting the test dose of ⁵⁹Fe in 100 ml of a 20% ethanol solution.

Two groups of 6 rabbits were given methotrexate intramuscularly (methotrexate sodium, Lederle Laboratories) for 6 days at doses of 0.5 mg/day or 1.6 mg/day. These last two groups had a normal diet.

Table 1. The Experimental Procedure

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Animals</th>
<th>Food</th>
<th>Drink</th>
<th>Drug Administered Intramuscularly</th>
<th>Duration of This Regime Before Experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>5</td>
<td>Normal</td>
<td>Water</td>
<td>—</td>
<td>10 wk</td>
</tr>
<tr>
<td>Controls</td>
<td>5</td>
<td>Folic acid deficient</td>
<td>Water</td>
<td>—</td>
<td>10 wk</td>
</tr>
<tr>
<td>Chronic alcohol</td>
<td>10</td>
<td>Normal</td>
<td>20% Ethanol solution</td>
<td>1 mg of folic acid/15 days</td>
<td>8 wk</td>
</tr>
<tr>
<td>Chronic alcohol</td>
<td>5</td>
<td>Normal</td>
<td>20% Ethanol solution</td>
<td>1 mg of folic acid/15 days</td>
<td>24 wk</td>
</tr>
<tr>
<td>Folic acid deficiency</td>
<td>10</td>
<td>Folic acid deficient</td>
<td>Water</td>
<td>—</td>
<td>10 wk</td>
</tr>
<tr>
<td>Folic acid deficiency</td>
<td>5</td>
<td>Folic acid deficient</td>
<td>20% Ethanol solution</td>
<td>—</td>
<td>10 wk</td>
</tr>
<tr>
<td>Alcohol</td>
<td>Acute alcohol</td>
<td>5</td>
<td>Normal</td>
<td>Water</td>
<td>10 wk</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>6</td>
<td>Normal</td>
<td>Water</td>
<td>Methotrexate, 0.5 mg/day</td>
<td>6 days</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>6</td>
<td>Normal</td>
<td>Water</td>
<td>Methotrexate, 1.6 mg/day</td>
<td>6 days</td>
</tr>
</tbody>
</table>

*Alcohol (20% ethanol solution) was administered at the same time as the radiolabeled dose test.
The ferrokinetics were performed 5 days after beginning the administration of methotrexate. The administration of this drug did not produce any alteration in the weight or consistency of feces. Food and liquids were supplied ad libitum in all groups.

Ferrokinetics

Iron absorption and PIT were measured on the same day. After an overnight fast, PIT was determined by intravenous injection of 1 ml of transferrin-bound $^{59}$Fe. This was prepared by incubating 1 ml of fresh rabbit plasma with 20 μCi of $^{59}$FeCl$_3$ (Swiss Federal Institute for Nuclear Research, Wurenlingen, Switzerland), which had been diluted in 0.005 N HCl and mixed with sufficient sterile 4% sodium citrate to obtain a molar ratio of citrate to iron in excess of 50:1. Five 2.5-ml samples of arterial blood were then obtained at 10, 30, 60, 90, and 120 min after injection of radiolabeled transferrin. PIT was calculated and expressed as nmole/kg/day. Plasma volume was determined in each animal by extrapolating the radioiron disappearance curve to zero and dividing the counts at time zero into the total counts injected. Blood volume was then derived from the plasma volume and the hematocrit. The $^{59}$Fe was determined by liquid scintillation counting, using the method of Eakins and Brown.

Prior to the administration of transferrin-bound $^{59}$Fe, we checked the alcoholemia and the level of folic acid in all rabbits. In every case, after 18 hr of fast (drink of alcoholic groups was replaced by water), the alcoholemia was zero, therefore excluding an action of alcohol on internal folate distribution.

Iron Absorption

In preliminary experiments, it was determined that the iron absorption from rabbit food labeled extrinsically was too low to measure. Iron absorption was measured beginning 1 hr after completion of the PIT determination. Drinking water was replaced with the test dose, which consisted of 10 μmole $^{59}$Fe$^{2+}$ as ferrous citrate (Swiss Federal Institute for Nuclear Research, total radioactivity of 1 μCi), 100 μmole L (+) ascorbate in 0.001 N HCl, and water to 100 ml. The addition of ascorbic acid to the test dose was done in order to increase iron absorption. In all cases, more than 90% of the test dose was consumed during the following 6 hr. The animals for an additional 12 hr after receiving the test dose. Initial radioactivity, contained in the rabbit and its feces collected during the administration of the test dose, and the final radioactivity, measured on day 14, were determined. The following formula was used to calculate the percent absorption of iron:

$$\text{Percent Absorption} = \frac{\text{cpm Day 14}}{\text{cpm Day 1}} \times 100 \times F$$

where cpm means counts per minute after subtraction of background, and $F$ is the correction factor for the radioactive decay of $^{59}$Fe, derived by comparison to a standard.

Radioactivity Measurements

A whole-body gamma spectrometer for animals was used. To maintain uniform counting geometry, animals were immobilized within a small rabbit cage. In order to measure the radioactivity contained in rabbit feces, it was necessary to correct the count rate for differences in body and feces configurations. A 2.5-liter plastic water bottle was used as a standard, to which $^{59}$Fe was added in an amount equal to half of the dose given to the animals. Thus, a factor (0.705) was determined that permitted the comparison of body and feces radioactivity.

Intestinal Transit Time

In some experiments, the first food administered after the radioactive iron test dose was stained with carmine red (6% water solution). The duration of the elimination of stained feces was recorded.

Hematologic and Serologic Determinations

Erythrocyte count (RBC), hematocrit (PCV), hemoglobin (Hb), and globular indices (MCV, MCH, and MCHC) were done with a laser ray counter (Hemac 630 L, Ortho Diagnostics Instruments, New Jersey). The spectrophotometric method, using bathophenantroline as reagent, suggested by the International Commission for Standardization in Hematology, was used for measuring serum iron. Reticulocytes (Ret) were counted in 2000 red cells stained with methylene blue and counterstained with...
Wright's stain. Serum folic acid levels were measured using a commercial radioassay (Diagnostic Products Corporation, Los Angeles, Calif.). In a previous work, we showed a good correlation between the serum folate levels in man measured by the radioassay and the microbiologic method using Lactobacillus casei. The levels of serum folate were measured by these two methods in rabbits from the normal group and the folic-acid-deficient group, showing a good correlation. Nevertheless, we preferred the radioassay, because in certain groups of rabbits, the alcohol or methotrexate can alter the microbiologic method more easily than the radioassay. Blood ethanol levels were determined by a modification of the alcohol dehydrogenase method. The serum alkaline phosphatase, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), and serum bilirubin were measured by routine laboratory methods.

Statistical Analysis

Statistical calculations were performed using the Student's t test for paired samples and for the comparison of means.

RESULTS

Evaluation of the Iron Absorption Method

The retention and elimination of $^{59}$Fe in five normal rabbits was measured after administration of the test dose (Fig. 1). The levels of radioactivity in the rabbit and its feces and urine were determined. The percent of body retention plus the percent elimination were calculated each day for each rabbit. The total was approximately 100%. At no time was the amount of radioactivity eliminated in the urine significantly different from the background. The body radioactivity decreased sharply the first 3 days (from 100% to 14.80% ± 3.75%). Between days 3 and 6, the rate of decrease was less. The body radioactivity remained constant after day 6 (3.69% ± 1.58% at day 15). As the body radioactivity decreased, the cumulative feces radioactivity increased until day 6. After this date, the feces no longer contained $^{59}$Fe. We found no correlation between the daily weight of feces and their radioactivity. The intestinal transit time was 24–72 hr when measured after a 12-hr fast following the radioiron test dose.

Effect of Ethanol Ingestion on Rabbits

After 2 or 6 mo of alcohol ingestion, the mean weight of the rabbits (4.2 ± 0.8 kg at 2 mo and 4.7 ± 0.6 kg at 6 mo) was not significantly different from controls.
Table 2. Hematologic Determinations

<table>
<thead>
<tr>
<th></th>
<th>Number of rabbits</th>
<th>Hb (g/dl)</th>
<th>PCV (%)</th>
<th>RBC (× 10^12/liter)</th>
<th>MCV (fl)</th>
<th>MCH (pg)</th>
<th>MCHC (g/dl)</th>
<th>Ret (%)</th>
<th>Serum folic acid (μg/liter)</th>
<th>Plasma iron (μmole/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>10</td>
<td>12.04 ± 2.06</td>
<td>38.71 ± 4.15</td>
<td>5.51 ± 1.00</td>
<td>70.14 ± 3.38</td>
<td>21.96 ± 0.57</td>
<td>32.51 ± 2.77</td>
<td>3.82 ± 1.26</td>
<td>40.23 ± 8.32</td>
<td>23.36 ± 3.07</td>
</tr>
<tr>
<td>Alcohol (2 mol)</td>
<td>10</td>
<td>12.10 ± 0.58</td>
<td>40.74 ± 1.63</td>
<td>5.36 ± 0.52</td>
<td>76.20 ± 1.79†</td>
<td>23.28 ± 0.89*</td>
<td>29.66 ± 0.66*</td>
<td>3.48 ± 1.01</td>
<td>36.08 ± 7.72</td>
<td>28.52 ± 4.76*</td>
</tr>
<tr>
<td>Alcohol (6 mol)</td>
<td>5</td>
<td>11.90 ± 0.35</td>
<td>40.64 ± 0.55</td>
<td>5.05 ± 0.18</td>
<td>75.20 ± 1.64*</td>
<td>23.60 ± 0.69†</td>
<td>33.62 ± 0.68</td>
<td>3.26 ± 0.86</td>
<td>39.78 ± 12.06</td>
<td>29.37 ± 6.51*</td>
</tr>
<tr>
<td>Folic Acid Deficiency</td>
<td>10</td>
<td>10.32 ± 1.52</td>
<td>30.78 ± 5.47*</td>
<td>3.54 ± 1.03†</td>
<td>82.13 ± 4.21‡</td>
<td>28.27 ± 0.86‡</td>
<td>33.82 ± 1.38</td>
<td>1.16 ± 0.26‡</td>
<td>8.48 ± 7.39‡</td>
<td>47.90 ± 9.06‡</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(12.11 ± 2.25)</td>
<td>(39.02 ± 3.48)</td>
<td>(5.63 ± 1.20)</td>
<td>(72.31 ± 5.61)</td>
<td>(22.33 ± 0.93)</td>
<td>(34.02 ± 2.65)</td>
<td>(4.21 ± 1.40)</td>
<td>(37.93 ± 10.35)</td>
<td>(21.38 ± 4.77)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.26 ± 1.68</td>
<td>31.12 ± 3.27*</td>
<td>3.84 ± 0.63*</td>
<td>79.82 ± 2.07‡</td>
<td>26.92 ± 0.54‡</td>
<td>33.08 ± 1.31</td>
<td>0.88 ± 0.31‡</td>
<td>9.18 ± 2.30‡</td>
<td>42.43 ± 5.33‡</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(12.81 ± 0.98)</td>
<td>(39.49 ± 5.21)</td>
<td>(5.81 ± 0.95)</td>
<td>(71.28 ± 2.29)</td>
<td>(22.40 ± 0.82)</td>
<td>(33.65 ± 0.75)</td>
<td>(4.82 ± 2.04)</td>
<td>(39.22 ± 7.17)</td>
<td>(22.55 ± 4.81)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12.52 ± 0.25</td>
<td>37.75 ± 2.21</td>
<td>5.42 ± 0.82</td>
<td>70.81 ± 2.37</td>
<td>22.43 ± 0.75</td>
<td>33.52 ± 2.01</td>
<td>3.63 ± 0.89</td>
<td>38.72 ± 9.20</td>
<td>22.85 ± 4.25</td>
</tr>
</tbody>
</table>

Values are mean ± SD.
*Significantly different from controls by unpaired t test, p < 0.01.
†Significant at p < 0.001.
‡Significant at p < 0.0001.
Values in parenthesis are those obtained after folic acid replacement (see experimental procedure).
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(4.8 ± 1.1 kg at 2 mo and 5.2 ± 0.9 kg at 6 mo). There was no serologic evidence of liver dysfunction as determined by SGOT, SGPT, serum alkaline phosphatase, and serum bilirubin. At the end of the experiment, the rabbits were killed. Histologic examination of the liver showed fatty degeneration with no evidence of cirrhosis.

Blood alcohol levels were checked 5 times during the experiment in each rabbit, showing values between 80 and 320 mg/dl. Finally, after acute or chronic alcohol ingestion, the quantity and consistency of feces were similar to those of controls.

Iron Absorption and PIT in the Control Group

In the two groups of five rabbits used as controls (one fed with normal rabbit food and the other with a folic-acid-deficient diet reconstituted with folic acid) the hematologic parameters (Hb, 11.98 ± 1.79 g/dl and 12.10 ± 2.21 g/dl; PCV, 37.22% ± 3.21% and 40.20% ± 3.80%; RBC, 5.42 ± 1.07 x 1012/liter and 5.60 ± 0.89 x 1012/liter; MCV, 71.03 ± 3.42 fl and 69.25 ± 4.01 fl; MCH, 21.58 ± 0.69 pg and 22.34 ± 0.71 pg; MCHC 31.93 ± 1.85 g/dl and 33.09 ± 2.53 g/dl; Ret 3.42% ± 0.91% and 4.22% ± 1.36%; serum folic acid 42.51 ± 9.35 µg/liter and 37.95 ± 10.71 µg/liter; plasma iron, 22.25 ± 1.26 µmole/liter and 24.47 ± 2.02 µmole/liter), iron absorption (2.46% ± 1.80% and 3.32% ± 1.27%), and PIT (10.02 ± 1.65 nmole/kg/day and 11.26 ± 1.97 nmole/kg/day) were nearly identical. For this reason, the two groups were treated as one for statistical analysis. The mean iron absorption of 10 control rabbits was 2.89% ± 1.57% and PIT was 10.64 ± 1.81 nmole/kg/day.

Effect of Chronic Alcohol Ingestion on Iron Absorption and PIT

The effect of alcohol ingestion during a period of 2 (10 animals) or 6 mo (5 animals) in rabbits not deficient in folic acid was tested. The Hb, PCV, RBC, Ret, and serum folic acid were normal (Table 2). The MCV, MCH, and serum iron were increased significantly. Macroovalocytosis and hypersegmentation of the white cells have been shown in the blood film. The PIT was slightly decreased (8.98 ± 1.16 nmole/kg/day for 2 mo and 8.53 ± 0.72 nmole/kg/day for 6 mo; p < 0.05) in the 2 groups, but the iron absorption was normal (3.24% ± 1.26% for 2 mo and 3.54% ± 2.01% for 6 mo; p > 0.50) (Fig. 2).

Fig. 2. Effect of chronic alcohol ingestion and/or folic acid deficiency on iron absorption and PIT. Open bars, mean iron absorption ± 1 SD; hatched bars, mean PIT ± 1 SD; in parentheses, the number of animals.
Effect of Folic Acid Deficiency on Iron Absorption and PIT

We studied the effect of folic acid deficiency on a group of 10 rabbits. This group did not show a significant difference in the Hb level, but the PCV, RBC, Ret, and serum folic acid were decreased, and the MCV, MCH, and serum iron were increased (Table 2). In this group, increased iron absorption (70.53% ± 13.76%) and PIT (58.35 ± 9.61 nmole/kg/day) were found (p < 0.0001) (Fig. 2). Two weeks after folic acid treatment, the iron absorption decreased (3.83% ± 1.87%) to a level similar to that of the control group (p > 0.50).

Effect of Folic Acid Deficiency and Chronic Alcohol Ingestion on Iron Absorption and PIT

In this experiment we used a group of five rabbits. The PCV was normal; the Hb, RBC, Ret, and serum folic acid decreased; and the MCV, MCH, and serum iron increased (Table 2). The PIT (11.52 ± 3.55 nmole/kg/day) was normal in comparison to the control group (p > 0.50), but the iron absorption (24.02% ± 2.81%) was increased (p < 0.0001). It was nevertheless lower than that found in the group with folic acid deficiency alone (p < 0.0001) (Fig. 2). Two weeks after treatment with folic acid, the iron absorption (3.33% ± 0.46%) was restored to normal (p > 0.50).

Effect of Acute Alcohol Ingestion on Iron Absorption

In one group of rabbits we administrated a 20% ethanol solution with radiolabeled iron. The various hematologic parameters did not show a significant difference from the controls (Table 2). However, the iron absorption (0.81% ± 0.24%) was significantly lowered in comparison to the control group (p < 0.02).

Effect of Methotrexate Administration on Iron Absorption and PIT

The effect of methotrexate administration was studied in two groups of 6 rabbits treated for 6 days. In the group given 0.5 mg/day of methotrexate, the MCH was increased (Table 3) while the serum folic acid decreased. In the group given 1.6 mg/day, the PCV and serum folic acid were decreased, and the MCV and MCH were increased. In both groups, the PIT was normal (p > 0.50) (Table 3). A dose–response effect of methotrexate on iron absorption was obtained.

Table 3. Effects of Methotrexate Administration on Rabbits

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Methotrexate 0.5 mg/Day*</th>
<th>Methotrexate 1.6 mg/Day*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>11.85 ± 1.17</td>
<td>11.10 ± 0.95</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>37.93 ± 3.39</td>
<td>35.43 ± 3.49</td>
</tr>
<tr>
<td>RBC (× 10¹²/μl)</td>
<td>5.59 ± 0.51</td>
<td>4.94 ± 0.53</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>69.33 ± 2.16</td>
<td>71.33 ± 1.97</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>21.15 ± 0.91</td>
<td>22.42 ± 0.62</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>31.18 ± 0.83</td>
<td>31.30 ± 0.95</td>
</tr>
<tr>
<td>Ret (%)</td>
<td>3.46 ± 1.05</td>
<td>3.30 ± 0.43</td>
</tr>
<tr>
<td>Serum folic acid (μg/μl)</td>
<td>40.15 ± 10.38</td>
<td>28.05 ± 5.71</td>
</tr>
<tr>
<td>Plasma iron (μmole/μl)</td>
<td>23.15 ± 3.98</td>
<td>25.39 ± 5.69</td>
</tr>
<tr>
<td>PIT (nmole/kg/day)</td>
<td>—</td>
<td>10.25 ± 1.38</td>
</tr>
<tr>
<td>Iron absorption (%)</td>
<td>—</td>
<td>6.64 ± 3.22</td>
</tr>
</tbody>
</table>

*Values are mean ± 1 SD.
†Methotrexate was given to 2 groups of 6 rabbits at the indicated dose for 6 days. Data were collected before and 5 days following methotrexate treatment.
‡NS, not significant; p > 0.05.
DISCUSSION

Various experimental animals have been used for studies on iron absorption.25 To our knowledge, in vivo iron absorption has not been studied in rabbits. Nevertheless, in vitro iron absorption studies in this animal have known similar control mechanisms as in other animals and in man. The iron absorption is increased in the iron-deficient rabbit and decreased in the iron-overloaded rabbit.26 Whole-body counter measures have been described as the most sensitive method for iron absorption studies.20,25 Modification of the test dose for iron absorption has allowed for detection of both increased and decreased absorption. Our evaluation of the use of a whole-body counter as an iron absorption method in rabbits has shown that the iron is eliminated in two phases (Fig. 1). The first, up to the third day, can be considered to correspond with the elimination of iron that did not pass through the mucosal wall. This is compatible with the intestinal transit time, which was from 1 to 3 days. The second phase, between days 3 and 6, could correspond with the radioiron that crossed the intestinal mucosal cell membrane, was fixed by intracellular ferritin, and returned to the intestinal lumen after desquamation of the mucosa.25,27

An excessive consumption of alcoholic beverages was implicated in the pathogenesis of hemosiderosis in alcoholic patients. Several authors28–30 have demonstrated that patients with liver cirrhosis have increased iron absorption, but the mechanism is not understood.

We have demonstrated in a group of rabbits a diminution of iron absorption when we administered the test dose simultaneously with an ethanol solution. This experiment suggests that in rabbits, acute alcohol ingestion has a similar effect on iron absorption as it has in man.10 Since acute alcohol ingestion did not increase iron absorption, the effect of chronic alcohol ingestion was tested. After 2 or 6 mo of chronic alcohol ingestion, macrocytosis developed in non-folic-acid deficient rabbits, probably secondary to a toxic effect of alcohol.31 The iron absorption in these two groups was normal. The decrease of PIT suggests a blockage of erythropoiesis. This effect of ethanol has been shown in man,32–34 and it is possible that it is due to a decrease of reticulocyte protein synthesis.35 In the group in which we induced a folic acid deficiency, the iron absorption as well as the PIT were increased. However, when folic acid deficiency developed in the presence of chronic alcohol ingestion, the iron absorption was still increased despite normal PIT. This suggests first, that alcohol may block the ineffective erythropoiesis induced by folic acid deficiency, and second, that the increased iron absorption is probably in connection with other mechanisms in which folic acid deficiency can interfere independently of the PIT. The decrease of iron absorption in the second group is probably directly related to the difference in PIT.

The methotrexate administration caused minor hematologic changes in two rabbit groups. The serum folic acid was decreased in proportion to the dose administrated. However, it is not known if this reflects a decrease in total body folic acid stores or is merely a depression of serum folate levels.34 The methotrexate is concentrated in the bile, and therefore, the gut is exposed to much higher levels than are other tissues.36 Subsequently, it is possible that the intestinal mucosal cells developed a local deficiency of folic acid. This fact, as in the previous experiments, could explain an elevated iron absorption that increases in proportion to the dose of methotrexate, despite a normal PIT.
Folic acid deficiency was first implicated in the pathogenesis of iron overload by MacDonald in 1966.\textsuperscript{37} In alcoholics, this hypothesis could explain many contradictions in the literature. Studies in rats chronically drinking an ethanol solution did not show an increase in iron absorption,\textsuperscript{38-40} perhaps because these animals did not develop a folic acid deficiency. On the other hand, the studies in man that have shown a normal iron absorption in alcoholics\textsuperscript{41-43} could have been done in patients who did not have a folic acid deficiency, as opposed to those studies that have shown an increase in iron absorption.\textsuperscript{38-39}

Sideroblastic or hemolytic anemias induced by alcohol\textsuperscript{44} can play a role in increased iron absorption by an increase of the PIT. We did not study this possibility. However, if alcohol can block the increased PIT secondary to a folic acid deficiency, it might also inhibit the effect of these two types of anemias on PIT.

These studies suggest that in our experimental situation the increased iron absorption is related to folic acid deficiency. Alcohol decreased iron absorption upon acute administration; chronically administered, it plays no direct role in iron absorption. Folic acid deficiency increases iron absorption by two probable mechanisms: first by increased PIT, which is associated with ineffective erythropoiesis, and second, by a probable direct effect on the intestinal mucosal membranes.

It is possible that if alcoholic beverages were fortified with folic acid, as has been suggested by Kaunitz and Lindenbaum,\textsuperscript{44} iron overload and its injurious effects would disappear.

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