Effect of starvation and protein depletion on ferrokinetics and iron absorption

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Iron deficiency is frequent in populations of limited means and is detected with greatest frequency during periods of maximal growth. Dietary-induced iron deficiency has been attributed to lack of iron in food, poor absorption of iron from certain foodstuffs, ingestion of iron-binding compounds, and dietary deficiency of porphyrin iron. In warmer climates increased loss of iron from body surfaces and a high incidence of hookworm and malabsorptive disorders may contribute significantly to the incidence and severity of iron deficiency (2, 3). Although there are many studies of dietary, mucosal, and intraluminal factors which affect iron absorption, there are few investigations of the effect of starvation or malnutrition on the absorption of iron. The prevalence of iron deficiency in malnourished populations (33), the marked alteration of iron kinetics in rats starved for erythropoietin assay (18), and different conclusions of the effect of starvation on iron absorption in previous animal studies (9, 11) led us to study the effect of starvation and dietary depletion of certain nutrients on iron absorption in rats.

MATERIALS AND METHODS

Male albino rats, Walter Reed Carworth Farms strain, weighing 100–225 g, were obtained and measurements of iron absorption and metabolism performed when normal control animals weighed between 225 and 275 g. The principles of laboratory animal care, as promulgated by the National Society for Medical Research, were observed. The standard laboratory diet contained 25% protein and 8–9 mg iron/100 g dry weight. Special diets in each experiment were made to contain equal quantities of iron by the addition of appropriate amounts of ferrous sulfate. Diets were assayed for iron content by a modification of the method of Lorber (20). Animals were made iron deficient by phlebotomy of 4 ml blood, 6 and 10 days before iron absorption studies were performed. Rats were iron loaded by intramuscular injection of 25 mg dextran-iron (Imferon) 2 weeks before studies were initiated. Hemolysis was induced by intramuscular injections of 10 mg acetylphenylhydrazine three times per week beginning 1 week before the initiation of studies.

Iron absorption studies were performed in rats fasted from food for various periods. Observations in 1-day-fasted rats were considered base-line values because recently ingested food decreases iron absorption and elevates the serum iron concentration; food iron dilutes test doses of radioiron within the intestinal lumen to act as additional carrier iron and recently absorbed iron is detected in the plasma (4, 11, 13, 23, 24, 28). Oral doses
were injected into the stomach through a 17-gauge olive-tipped endoesophageal needle. Test doses were prepared immediately before dosing to contain 0.5 μg ferrous 59 citrate (Abbott, 0.6 μg/mc) and 0.25 mg iron as commercial ferrous sulfate (Vitarine Corp.) in 1 ml distilled water. Absorption of the radioiron was quantified by measurement of total body radioactivity in a small animal, whole-body liquid-scintillation detector (Packard ARMAC) Whole-body radioactivity (0.8 Mev–∞) was measured at 3 hr and at 7 days after the oral dose of radioiron 59 to determine the percent of the tracer absorbed by rats. Standards were prepared for each experiment by measuring an identical test dose of iron 59 into a 250-ml water-filled plastic bottle. The animals, restrained in vented quart ice cream cartons, were counted and compared to the reference standard to determine the quantity of radioiron retained by each animal. The percent retained is equivalent to the quantity absorbed from test doses (11).

Certain rats were killed at intervals after intragastric doses of iron 59 (0.25 mg) or intravenous injection of plasma labeled with radioiron. The abdomen was opened through a ventral incision and the intestinal tract was aspirated in vented quart ice cream cartons, were counted and compared to the reference standard to determine the quantity of radioiron absorbed by rats. Standards were prepared for each experiment by measuring an identical test dose of iron 59 into a 250-ml water-filled plastic bottle. The animals, restrained in vented quart ice cream cartons, were counted and compared to the reference standard to determine the quantity of radioiron retained by each animal. The percent retained is equivalent to the quantity absorbed from test doses (11).

TABLE 1. Effect of starvation on iron absorption and factors related to iron kinetics

<table>
<thead>
<tr>
<th>Period of Fast</th>
<th>Loss of Wt</th>
<th>Hct</th>
<th>Absorp. of Iron</th>
<th>Serum Iron Concen</th>
<th>Total Iron Binding Capacity</th>
<th>Plasma 89Fe Clearance To</th>
<th>Ra. iron Turnover</th>
<th>RBC Incorpor. 89Fe</th>
<th>Small Intestinal Iron</th>
<th>Hepatic Iron</th>
<th>Chemical Assay</th>
<th>Chemical Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days</td>
<td>%</td>
<td>%</td>
<td>μg/100 ml</td>
<td>min</td>
<td>83</td>
<td>533</td>
<td>42.0</td>
<td>1.92</td>
<td>20.4</td>
<td>15.4</td>
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<td>49.1</td>
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<td>490</td>
<td>59</td>
<td>135</td>
<td>42.0</td>
<td>1.92</td>
<td>20.4</td>
<td>15.4</td>
<td>10.6</td>
<td>656</td>
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<td></td>
<td>±0.8</td>
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<td>105</td>
<td>43.8</td>
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<td>±9.6</td>
<td>±12.1</td>
<td>±2.3</td>
<td>±9.0</td>
<td>±1.4</td>
<td>±0.11</td>
<td>±1.1</td>
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<td>490</td>
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<td>12.9</td>
<td>650</td>
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<tr>
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<td>±1.7</td>
<td>±8.1</td>
<td>±8.9</td>
<td>±4.2</td>
<td>±5.6</td>
<td>±0.9</td>
<td>±0.24</td>
<td>±0.9</td>
<td>±0.8</td>
<td>±0.6</td>
<td>±13.4</td>
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<td>3</td>
<td>52.4</td>
<td>16.1</td>
<td>150</td>
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<td>109</td>
<td>90</td>
<td>11.5</td>
<td>3.50</td>
<td>12.5</td>
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<td>14.7</td>
<td>658</td>
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<td>±0.9</td>
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<td>±5.5</td>
<td>±11.4</td>
<td>±5.6</td>
<td>±6.7</td>
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<td>±0.39</td>
<td>±0.7</td>
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<td>±17.9</td>
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<td>4</td>
<td>54.7</td>
<td>10.2</td>
<td>182</td>
<td>445</td>
<td>133</td>
<td>90</td>
<td>4.1</td>
<td>4.50</td>
<td>12.0</td>
<td>13.8</td>
<td>16.8</td>
<td>627</td>
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<tr>
<td></td>
<td>±1.2</td>
<td>±1.2</td>
<td>±9.6</td>
<td>±9.4</td>
<td>±6.9</td>
<td>±4.1</td>
<td>±0.4</td>
<td>±0.30</td>
<td>±0.8</td>
<td>±0.9</td>
<td>±0.8</td>
<td>±15.7</td>
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<tr>
<td>5</td>
<td>56.6</td>
<td>5.4</td>
<td>221</td>
<td>420</td>
<td>177</td>
<td>80</td>
<td>3.1</td>
<td>4.94</td>
<td>12.9</td>
<td>15.4</td>
<td>17.2</td>
<td>638</td>
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<tr>
<td></td>
<td>±1.1</td>
<td>±0.6</td>
<td>±12.6</td>
<td>±8.6</td>
<td>±9.1</td>
<td>±5.2</td>
<td>±0.4</td>
<td>±0.41</td>
<td>±1.0</td>
<td>±0.6</td>
<td>±0.8</td>
<td>±18.4</td>
</tr>
</tbody>
</table>

* Day 1 of fast should be used for base-line comparison because day 0 values reflect the recent ingestion of food.

were injected into the stomach through a 17-gauge olive-tipped endoesophageal needle. Test doses were prepared immediately before dosing to contain 0.5 μg ferrous 59 citrate (Abbott, 0.6 μg/mc) and 0.25 mg iron as commercial ferrous sulfate (Vitarine Corp.) in 1 ml distilled water. Absorption of the radioiron was quantified by measurement of total body radioactivity in a small animal, whole-body liquid-scintillation detector (Packard ARMAC) Whole-body radioactivity (0.8 Mev–∞) was measured at 3 hr and at 7 days after the oral dose of radioiron 59 to determine the percent of the tracer absorbed by rats. Standards were prepared for each experiment by measuring an identical test dose of iron 59 into a 250-ml water-filled plastic bottle. The animals, restrained in vented quart ice cream cartons, were counted and compared to the reference standard to determine the quantity of radioiron retained by each animal. The percent retained is equivalent to the quantity absorbed from test doses (11).

Certain rats were killed at intervals after intragastric doses of iron 59 (0.25 mg) or intravenous injection of plasma labeled with radioiron. The abdomen was opened through a ventral incision and the intestinal tract was excised, unopened. The proximal quarter of the small intestine was isolated, opened lengthwise, and washed in five changes of iron-free water (pH 8.0). Organs were prepared for measurement of total radioactivity by adding concentrated HCl and diluting the digest to 250 ml in water-filled plastic bottles. Gut and liver specimens were prepared for iron analysis in a Virtis tissue homogenizer. The nonheme iron content was measured by a modification of the method of Brückmann and Zondek (5, 12). Protein-bound iron was separated from relatively available iron by homogenization of washed intestinal segments in a 0.5 M tris (pH 9.0) solution (tris(hydroxy-

* Only negligible quantities of radioiron were detected in fecal collections. Numbers do not total to 100% because the fenumis are only an aliquot of buse marrow and only representative organs were selected for measurement of radioactivity.

The pH of duodenal aspirates was measured with a calibrated Leeds and Northrup pH meter. Precipitation was defined as the quantity of radioiron in aspirates which was retained by a 0.45-μ Millipore filter. The molecular size of the radioiron in the filtrates was measured at 3-7 days after the oral dose of radioiron 59 to determine the percent of the tracer absorbed by rats. Standards were prepared for each experiment by measuring an identical test dose of iron 59 into a 250-ml water-filled plastic bottle. The animals, restrained in vented quart ice cream cartons, were counted and compared to the reference standard to determine the quantity of radioiron retained by each animal. The percent retained is equivalent to the quantity absorbed from test doses (11).

TABLE 2. Percent of 89Fe in various organs 24 hr after an iv dose

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Fasted 5 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duodenum</td>
<td>1.47±0.69</td>
<td>3.94±1.67</td>
</tr>
<tr>
<td>Fenumis</td>
<td>4.04±0.71</td>
<td>5.64±0.64</td>
</tr>
<tr>
<td>Liver</td>
<td>13.64±1.26</td>
<td>19.23±1.69</td>
</tr>
<tr>
<td>Spleen</td>
<td>3.26±0.21</td>
<td>1.11±0.16</td>
</tr>
<tr>
<td>Kidneys</td>
<td>2.40±0.15</td>
<td>3.17±0.52</td>
</tr>
<tr>
<td>Blood</td>
<td>53.63±3.46</td>
<td>8.62±1.15</td>
</tr>
</tbody>
</table>

* Only negligible quantities of radioiron were detected in fecal collections. Numbers do not total to 100% because the fenumis are only an aliquot of buse marrow and only representative organs were selected for measurement of radioactivity.
used to estimate the amount of iron in duodenal filtrates with a molecular weight greater than 700 (G-10) or 4,000 (G-25). The radioactivity in eluates and that retained by the Millipore filter and the gel filtration media was measured with a whole-body liquid-scintillation detector in water filled 250 ml plastic bottles.

Blood for iron measurements and determinations of the total iron-binding capacity was obtained from the retro-orbital venous plexus of rats with heparinized capillary tubes. The serum iron and unsaturated iron-binding capacity were determined by the "one-tube method" (31).

Plasma clearance studies were performed following the intravenous injection of iron 59-labeled rat serum. The ferrous 59 citrate was incubated with pooled rat serum for 15 min at 37 C before injection of 0.25 ml into the dorsal vein of the penis. The iron-binding capacity of the incubated serum was not exceeded. At 10, 20, 30, and 40 min following the injection, 0.02 ml whole blood was obtained from the tail vein of the rat with a hemoglobin pipette. The blood was diluted into 2 ml water and the radioactivity in each specimen was measured in a well-type crystal-scintillation detector (Packard auto gamma spectrometer, model 410A). The plasma iron clearance (T1) was determined from the best-fitted plot of these points on semilogarithmic graph paper. The plasma volume was calculated by extrapolating the plot of plasma clearance to zero time. Daily iron turnover was calculated from the serum iron concentration, plasma volume, and plasma iron clearance studies (2, 3). The incorporation of iron 59 or iron 55 into red blood cells was determined by measurement of the radioactivity in blood specimens obtained 90 hr after intravenous injection of labeled plasma (2, 3). Specimens containing iron 55 were prepared for measurement of radioactivity in a liquid-scintillation detector (Packard Tri-Carb) (21).

The red blood cell mass was calculated from the dilution of transfused homologous washed erythrocytes labeled with sodium chromate (S4Cr) (17). The packed cellular volumes were measured by the microhematocrit method and hemoglobin concentration by the cyanomethemoglobin technique. Red blood cells were enumerated in a Coulter counter (32).

Segments of small intestine were prepared for radioautography and histologic examination as described previously (8, 17). Oral glucose tolerance tests were performed after the intragastric administration of 1 g glucose. The serum glucose concentration was determined by the method of Nelson (22).

Values in the tables are reported as the means ± standard errors for 9 or 10 rats.

RESULTS

Starvation of normal iron-replete animals (Table 1). The effects of starvation on iron metabolism were studied in rats at intervals after food was removed from the animal cages. Observations in 1-day-fasted animals were considered base line. Iron absorption became significantly decreased after 3 days of starvation and was markedly diminished after a more prolonged fast. Prior to the decrease in iron absorption, starved rats showed: 1) loss of body weight; 2) an increased packed cellular volume; 3) decreased incorporation of iron into circulating erythrocytes; and 4) increased incorporation of an intravenous dose of radioiron into the small intestine and liver (day 2). Coincident with significant decreases in iron absorption, there was alteration of the serum iron concentration and transport through the plasma. The total iron-binding capacity became significantly decreased on the 4th day of starvation. Increases in the concentration of iron in the liver were caused by loss of weight from this organ. There was no increase in either the iron content or concentration of small intestinal specimens during the 5 days of starvation.

Incorporation of 59Fe into body organs (Table 2). Plasma

<table>
<thead>
<tr>
<th>Group</th>
<th>Iron Absorption, % Oral Dose</th>
<th>RBC Incorp. of Iron, % iv Dose</th>
<th>Hematocrit, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>20.6±2.3</td>
<td>32.0±1.2</td>
<td>48.2±0.7</td>
</tr>
<tr>
<td>Unfasted</td>
<td>22.5±2.1</td>
<td>32.9±1.0</td>
<td>48.8±0.9</td>
</tr>
<tr>
<td>Fasted 16 hr</td>
<td>6.0±0.8</td>
<td>4.1±0.2</td>
<td>57.1±1.1</td>
</tr>
<tr>
<td>(1-6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(7-8)</td>
<td>12.9±1.0</td>
<td>14.0±1.0</td>
<td>31.0±1.0</td>
</tr>
<tr>
<td>(9-11)</td>
<td>16.3±1.4</td>
<td>19.4±0.9</td>
<td>50.8±1.1</td>
</tr>
<tr>
<td>(12-14)</td>
<td>26.1±2.6</td>
<td>32.9±1.6</td>
<td>47.7±0.6</td>
</tr>
<tr>
<td>Normal</td>
<td>27.7±3.1</td>
<td>34.7±1.1</td>
<td>46.0±0.6</td>
</tr>
</tbody>
</table>

* Normal fasted 16-hr animal values should be used for baseline comparison because normal unfasted values reflect the recent ingestion of food.

TABLE 4. Rats studied at intervals after a 5-day period of starvation

* Rats received 2 g dextrose by hypodermoclysis daily.

TABLE 3. Effect of hydration on iron absorption

<table>
<thead>
<tr>
<th>Water intake, ml/day</th>
<th>Normal</th>
<th>Starved, 5 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food, g/day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wt gain or loss, g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hb Cr RBC mass, ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fe absorption, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RBC incorporation, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Starved, 5 Days</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Rats received 2 g dextrose by hypodermoclysis daily.
labeled with $^{55}\text{Fe}$ was injected intravenously into normal animals and rats starved for 5 days. Various organs were excised 24 hr later for measurement of radioactivity. Starved animals had significantly more radioiron in the small intestine, liver, bone marrow, and kidneys and less in red blood cells and the spleen. The increased incorporation of $^{55}\text{Fe}$ into organs of starved animals was probably caused by decreased erythropoiesis and diminished incorporation of iron into red blood cells (18).

**Erythremia and dehydration (Table 3).** The elevated hematocrit and unchanged red blood cell volume in starved animals indicated that there was a decreased plasma volume (9). This relative erythremia was probably caused by dehydration because our starved rats ingested less than half the volume of the water consumed by normal animals. To determine if relative erythremia or dehydration affected iron absorption, water was removed from the animal cages and rats were hydrated by hypodermoclysis of 10 ml 5 % dextrose at 6-hr intervals for 5 days. Half the animals were starved and the remainder were fed dried rat biscuits. The hematocrit remained normal in the parenterally hydrated starved animals but iron absorption and the red blood cell incorporation of $^{55}\text{Fe}$ was markedly depressed.

**Recovery from starvation (Table 4, Fig. 1).** Rats were starved for 5 days at intervals before the administration of oral test doses of $^{55}\text{Fe}$ and intravenous doses of plasma labeled with $^{55}\text{Fe}$. The animals were weighed daily and the mean weight of each group of 10 rats is illustrated in Fig. 1. Changes in iron absorption coincided with the occurrence of an increased packed cellular volume and decreased red blood cell incorporation of radioiron. Iron absorption became normal in rats which had 6 days to recover from the 5-day fast; this coincided with the period required for animals to regain their prefasted weight.

**Starvation in various states of iron repletion and hemolysis (Table 5, Fig. 2).** Normal, iron-replete rats had a fourfold decrease in iron absorption after 5 days of starvation (4.7 vs. 19.9 %). Iron deficient and acetylphenylhydrazine-treated rats absorbed more iron than normal animals (48.2 and 44.7 vs. 19.9 %). Starvation reduced the quantity of iron absorbed by iron-deficient and acetylphenylhydrazine-treated rats to amounts similar to that absorbed by normal unstarved animals (24.3 and 23.6 vs. 19 %). Starvation of iron-loaded rats caused only a slight decrease in the absorption of iron (4.9 vs. 6.7 %). Measurements of factors affecting iron metabolism in normal and iron-deficient unstarved animals were similar to studies reported previously (11). In iron deficient rats starvation decreased iron absorption, red cell incorporation of iron, and the concentration of plasma iron-binding protein, and increased the packed cellular volume, serum iron concentration, transport of iron through plasma, and the iron concentration in intestinal specimens and
The total quantity of iron in the gut and liver remained unchanged but was significantly less than that observed in specimens from normal, iron-replete animals. Intestinal uptake and absorption of iron (Tables 6, 7). Normal and iron-deficient rats, fasted either 1 or 5 days, were killed following an oral dose of radioiron. The intestinal tract was excised and radioactivity was measured in the carcass and washed duodenal segments. Specimens from the 1-day-fasted rats contained significantly more iron than specimens from starved animals. This suggested that starvation decreased the uptake of iron from the intestinal lumen into the duodenal mucosal cells. It has been postulated that the iron content of intestinal cells regulated the quantity of iron absorbed from the gut (8). To investigate this hypothesis in starved animals, we injected 56Fe-labeled plasma into rats and measured the radioiron in duodenal segments 20 hr later. Increased amounts of radioiron were incorporated into the intestinal segments of starved animals. Much of this iron was dialyzable and believed to be either unbound or easily split from protein.

Physical measurements of intraluminal iron (Table 8). Rats fasted 1 day or starved 5 days were killed 1 hr after an oral dose of radioiron. The duodenal contents from each animal were collected with a hypodermic needle and syringe, and aspirates from three rats were pooled. The pH was measured and the aspirates were injected through 0.45 μm bacterial filters. Aliquots of the filtrate were added to G10 and G25 gel filtration columns. The quantity of 56Fe eluted from columns in the first displacement volume provided a measurement of iron in test doses that form aggregates with a molecular size greater than substances with a molecular weight of 700 (G10) or 4,000 (G25). Similarity of findings in duodenal aspirates from normal and starved animals suggested that intraluminal factors such as pH, valence, and chelation were not the major cause of decreased absorption of iron by starved animals.

Intestinal turnover, histology and absorption of glucose. Histologic studies of the small intestine and measurements of mucosal lifespan were performed to ascertain if starvation caused changes which affected iron absorption. Tritium-labeled thymidine was injected intravenously into normal animals and rats which were starved for 5 days. The duodenum and jejunum were excised from these animals 24, 36, 40, 44, and 48 hr later. Radioautographs were prepared from sections of these guts and showed a slower rate of movement of labeled epithelial cells in the starved animals. In normal animals, radioactivity was visualized in epithelial cells in the extrusion zone at the tips of the villi at 40 hr, whereas the labeled mucosal cells of starved rats did not reach the villous tips until 48 hr after injection. Hematoxylin and eosin-stained sections of the duodenum and jejunum showed a normal villous architecture. Absorption of glucose was measured in normal and starved rats to ascertain if starvation caused generalized malabsorption. The blood sugar was measured in rats fasted overnight (75 mg/100 ml) or 5 days (60 mg/100 ml) before the oral administration of 1 g glucose and again 1/2 hr later. The increased concentration of glucose in the serum of both normal

![Graph showing absorption of iron in different states of iron repletion and hemolysis.](Image)

**Table 6. Percent of 56Fe in intestinal segments and carcass at intervals after an oral dose**

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Normal</th>
<th>Iron-Deficient</th>
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<tbody>
<tr>
<td>1</td>
<td>4.96±0.16/2.43±0.09</td>
<td>5.49±0.22/5.09±0.17</td>
</tr>
<tr>
<td></td>
<td>9.92±0.34/4.10±0.21</td>
<td>27.56±1.05/10.54±0.71</td>
</tr>
<tr>
<td></td>
<td>14.88/6.83</td>
<td>32.05/23.63</td>
</tr>
<tr>
<td>2</td>
<td>2.21±0.11/1.37±0.06</td>
<td>1.73±0.10/1.50±0.07</td>
</tr>
<tr>
<td></td>
<td>15.05±0.36/5.11±0.34</td>
<td>29.22±1.14/22.62±0.95</td>
</tr>
<tr>
<td></td>
<td>17.26/6.48</td>
<td>30.95/24.12</td>
</tr>
</tbody>
</table>
Body retention of a parenteral dose of radioiron $^{59}$ was measured twice weekly in rats fed diets containing either 27%, 8%, or no protein. The animals were fed the special diets for 2 weeks before they were injected with an intravenous dose of $^{59}$Fe-labeled plasma. Whole-body radioactivity was measured in a small-animal liquid-scintillation detector and by assay of the radioiron in stool collections (6). Rats fed the 27% protein diet lost 4% (SE 0.9%) of the $^{59}$Fe during the week after injection and subsequently excreted 0.2% of the parenteral dose daily. Animals fed a low-protein diet or a protein-depleted diet lost significantly more radioiron both during the 1st week after injection (B ± 1.1 and 16 ± 1.6%) and later (0.3 and 0.4%). Half of the animals fed a protein-depleted diet died during the last 2 weeks of study. At the termination of the study, blood specimens were obtained from each animal to characterize the anemia. Rats fed an 8% protein diet developed a moderate anemia, whereas lack of dietary protein caused a marked normocytic normochromic anemia.

**Rice and starch diets (Table 11).** Cooked white rice and diets containing either sucrose or corn starch were prepared for rats. Vitamin-free casein was added to control diets and corn oil, vitamins, and iron were added to each diet. The rats weighed 125 g at the beginning of the study and were kept on each diet for 3 weeks. Rats on protein-deficient diets gained less weight, absorbed less $^{59}$Fe from oral test doses of ferrous sulfate and incorporated less of an intravenous dose of $^{59}$Fe into red blood cells. To ascertain whether starch had a direct intraluminal effect on the absorption of iron, we fasted normal rats for 24 hr and gave them 5-ml oral doses of $^{59}$Fe containing either 1 g starch, 1 g sucrose, or no additive. The normal control animals absorbed 15.6% (SE 1.3%) of the test dose of iron. Rats dosed with the starch suspension and the sucrose solution absorbed 18.3% (SE 2.0%) and 16.4% (SE 1.8%) of the test dose of radioiron, respectively.
TABLE 9. Effect of dietary composition on iron absorption and factors related to iron kinetics

<table>
<thead>
<tr>
<th>Content of Diet, %</th>
<th>Mean Wt</th>
<th>Hematocrit</th>
<th>Absorption of Iron</th>
<th>Serum Iron Concentration</th>
<th>Total Iron Binding Capacity</th>
<th>Plasma 56Fe Binding Capacity</th>
<th>Calculated Iron Turnover</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal diet 27</td>
<td>233</td>
<td>45.5 ± 0.7</td>
<td>12.7 ± 1.9</td>
<td>103 ± 8.4</td>
<td>482 ± 18.1</td>
<td>71 ± 4.6</td>
<td>113 ± 6.7</td>
</tr>
<tr>
<td>Decreased protein diet 18</td>
<td>205</td>
<td>46.7 ± 0.3</td>
<td>11.6 ± 1.3</td>
<td>118 ± 9.3</td>
<td>462 ± 15.9</td>
<td>40.3 ± 1.5</td>
<td>102 ± 6.8</td>
</tr>
<tr>
<td>Low-protein diet</td>
<td>167</td>
<td>45.0 ± 1.0</td>
<td>4.5 ± 0.3</td>
<td>160 ± 10.2</td>
<td>410 ± 16.3</td>
<td>90 ± 7.3</td>
<td>102 ± 8.6</td>
</tr>
<tr>
<td>Protein-depleted diet 0</td>
<td>105</td>
<td>47.1 ± 0.9</td>
<td>3.0 ± 0.3</td>
<td>240 ± 14.8</td>
<td>365 ± 9.5</td>
<td>145 ± 5.2</td>
<td>59 ± 3.3</td>
</tr>
<tr>
<td>Fat-free diet</td>
<td>919</td>
<td>46.0 ± 0.9</td>
<td>3.9 ± 1.7</td>
<td>106 ± 7.7</td>
<td>485 ± 17.2</td>
<td>41.5 ± 1.2</td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION

Starvation causes profound alteration of body physiology and metabolism and has been used as a method to assay erythropoietin (15, 19). Many investigations of factors affecting erythropoiesis showed that starved animals developed a decrease red blood cell incorporation of iron and delayed plasma iron clearance (9, 18). The development of similar alterations of iron metabolism in animals fed a protein-depleted diet suggested that calorie starvation was less important than either impaired growth or protein deprivation (26, 27).

Protein deprivation causes weight loss or a retarded growth rate with a marked decrease in the body requirement for iron and hemoglobin synthesis. The total-body iron of a 200-g rat is approximately 12 mg, of which about half is found in the hemoglobin of circulating erythrocytes. Rats fed a normal diet gain approximately 2 g daily and must absorb at least 120 μg iron to maintain a total-body iron concentration of 60 ppm. Contrariwise, weight loss must be accompanied by a comparable loss of body iron or siderosis develops. Organ iron overloading is enhanced by significant suppression of erythropoiesis; a 200-g rat degrades about 22 mg hemoglobin daily containing 75 μg iron, and iron excretion from these animals is limited to approximately 50 μg/day (6). It is possible that these excessive accumulations of iron in one or more body organs were responsible for the changes in iron absorption and excretion.

Protein deprivation causes a decrease in organ size and this increases the concentration of iron within these tissues. The body attempts to re-establish a normal iron concentration by decreasing absorption and increasing excretion of iron. The marked diminution in erythropoiesis limits the amount of iron incorporated into red blood cells, resulting in increased storage of iron in other organs of the body (9). Although the red blood cell incorporation of iron is markedly decreased, the reduction in daily iron turnover is proportionate to changes in the body weight of animals.

It has been postulated that iron absorption is regulated by the iron content of intestinal cells and many experiments show an inverse relationship between the quantity of iron absorbed and the iron content or concentration in intestinal specimens (6, 13). In contrast, both starved animals and rats fed protein-depleted diets had a decreased iron content and unchanged iron concentration in duodenal specimens. It seemed unlikely that generalized malabsorption or the rate of intestinal cellular proliferation caused the fourfold decrease in iron absorption that was observed in starved animals, because these rats absorbed normal quantities of glucose and there was only a 20% delay in the turnover of intestinal epithelial cells (7, 8, 16). However, there was increased incorporation of body iron into the intestinal cells of starved animals and much of this iron seemed to be...
TABLE 11. Effect of rice and starch diets on iron absorption

<table>
<thead>
<tr>
<th>Additives to diet, g/100 g</th>
<th>Normal Diet (Starch)</th>
<th>Normal Diet (Sucrose)</th>
<th>Rice</th>
<th>Starch Casein</th>
<th>Starch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>27</td>
<td>27</td>
<td>5</td>
<td>01</td>
<td>06</td>
</tr>
<tr>
<td>Starch</td>
<td>60</td>
<td>60</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Corn oil</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dietary composition, g/100 g</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>25</td>
<td>25</td>
<td>8</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Chos</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Iron</td>
<td>0.008</td>
<td>0.008</td>
<td>0.008</td>
<td>0.008</td>
<td>0.008</td>
</tr>
<tr>
<td>Mean wt of rats, g</td>
<td>230</td>
<td>224</td>
<td>197</td>
<td>187</td>
<td>134</td>
</tr>
<tr>
<td>Iron absorption, %</td>
<td>18.2</td>
<td>17.6</td>
<td>9.6</td>
<td>6.8</td>
<td>2.8</td>
</tr>
<tr>
<td>%</td>
<td>±1.6</td>
<td>±2.3</td>
<td>±1.3</td>
<td>±0.9</td>
<td>±0.2</td>
</tr>
<tr>
<td>RBC incorporation of 59Fe, %</td>
<td>41</td>
<td>40</td>
<td>30</td>
<td>28</td>
<td>4</td>
</tr>
<tr>
<td>±1.4</td>
<td>±1.7</td>
<td>±1.3</td>
<td>±1.0</td>
<td>±0.1</td>
<td></td>
</tr>
</tbody>
</table>

A commercial salt mixture and vitamins were added to all diets. The composition of the rice diet is for uncooked white rice. Cooked rice becomes 70% hydrated. Values shown with ± SE are means for 10 rats.

FIG. 3. Excretion of iron from rats fed diets containing various amounts of protein was quantified by periodic measurements of the body retention of a parenteral dose of radioiron and by loss of 59Fe in stool. Protein depletion caused increased loss of parenteral iron from animals. Each point represents mean for a group of 10 animals.

either unbound or loosely bound to protein. That the body iron sequestered by intestinal epithelial cells is a more potent regulator of absorption than dietary iron was suggested in previous experiments, an intravenous dose of iron markedly decreased absorption (6), whereas the ingestion of carrier iron several hours before an oral test dose of radioiron only slightly reduced the quantity absorbed (4).

In starved animals and rats fed protein-deficient diets, several factors must be considered as possible regulators of the changes in ferrokinetics and erythropoiesis: 1) Erythremia and hemoconcentration are unlikely causes because abnormalities persist after anemia occurs and parenteral infusions that hydrate the animals fail to correct the ferrokinetic changes. 2) Decreased availability of amino acids for hemoglobin synthesis may be a contributory factor. However, Reissman showed that injections of erythropoietin into rats fed a protein-depleted diet prevented anemia and, if these animals and normal rats were hypertransfused, they had quantitatively similar incorporation of iron into red blood cells in response to hypoxia (26, 27). 3) The sequence of changes observed in starved rats and the disproportionate reduction of the red cell incorporation of iron in comparison to other ferrokinetic abnormalities suggested that the diminished erythropoiesis was not caused by unavailability of iron. 4) If decreased erythropoiesis is the stimulus for the ferrokinetic changes, erythropoietin may play an important role. However, the decreased levels of erythropoietin observed in starved animals are not caused by an inability to synthesize this hormone because these animals respond to iron deficiency or hemolysis, and erythropoietin levels are increased in hypoxic rats fed a protein-depleted diet (26, 27).

Our protein-deficient diets were similar to the food consumed by "starch eaters" and the inhabitants of rice-eating countries, except they contained adequate amounts of added iron (10, 29, 30). Our diets caused a normocytic, normochromic anemia, with decreased absorption of iron that was related to diminished erythropoiesis and not intraluminal intestinal factors. If iron was not added to our diets, the anemia would be complicated by iron deficiency because of the lack of iron in both commercial starch and rice. Although the relationship of our observations in rats to man is not known, it is tempting to postulate that protein depletion may contribute to both the severity of anemia and iron deficiency in malnourished humans.

REFERENCES
FERROKINETICS IN STARVATION AND PROTEIN DEPLETION 565


