IRON ABSORPTION is enhanced by iron deficiency in human subjects (see Josephs,\(^1\) Callender,\(^2\) and Moore\(^3\)), and in experimental animals (e.g., Bothwell, Pirzio-Biroli and Finch\(^4\)). The mechanism by which iron deficiency and other factors influence iron absorption by the small intestine has not yet been satisfactorily explained. The attractive hypothesis of “mucosal block,”\(^5\)-\(^8\) which might provide an explanation of the effect of iron deficiency, has been seriously criticized (see Moore\(^3\)) but no alternative hypothesis is available.

The experiments described here were undertaken to study the absorption of Fe\(^{59}\)-tagged iron salts by rats maintained on semisynthetic diets which produce an apparently uncomplicated state of iron deficiency.\(^9\) Results confirm the remarkable increase in absorption produced by iron deficiency and enable some suggestions to be made about mechanisms of absorption.

**Materials and Methods**

Female Wistar-strain albino rats were caged and fed as described previously.\(^9\)\(^-\)\(^11\) Weights and haemoglobin and hematocrit values were measured regularly.\(^9\) The iron-deficient groups were given diet 1A (the basic diet 1 of McCall et al.\(^9\) containing 2 mg. Fe per Kg. diet) for the first 4 to 6 weeks after weaning. The majority were then maintained on diet 1B (the basic diet supplemented with ferrous ammonium sulphate to provide 4 mg. Fe per Kg. diet), although small numbers were kept on diet 1A for comparison. Their iron-supplemented litter mates were fed on diet 2 (240 mg. Fe per Kg.) throughout. A small number of male rats were also used; they were maintained on a commercial cubed diet (41B,\(^12\)\(^-\)\(^13\) containing 95 mg. Fe per Kg.\(^9\)).

Iron absorption tests were carried out at arbitrary times between 3 and 10 months of age, after the phase of most rapid growth was over, most being carried out at 4 and 6 months of age. They were usually made on litter-mate pairs of iron-deficient and iron-supplemented rats, but some results have been combined into subgroups comprising members of several litters. Representative animals were killed at approximately 6 months of age for histologic studies not reported here and to provide blood samples for serum iron determination by a dipyridyl method.\(^14\)

Fe\(^{59}\) was obtained from the Radiochemical Centre, Amersham, as ferric chloride of high specific activity (1 to 5 \(\mu\)g. per \(\mu\)g.) in 0.1 N HCl and made up in two solutions for dosing:

1. **Ferrous**—Freshly prepared solutions of ferrous ammonium sulphate were added to the tracer Fe\(^{59}\)-ferric chloride solution so as to dilute it 10 times and bring the final pH to approximately 2. Isotope exchange between Fe\(^3^+\) and Fe\(^2^+\) may be assumed to occur in such solutions\(^15\)\(^,\)\(^16\) so that both fractions become uniformly labelled with Fe\(^{59}\); the validity of this assumption was confirmed by means of a combined o-phenanthroline and KSCN test system.\(^17\)

2. **Ferric**—Further ferric chloride solution was added to the tracer solution so as to dilute it 10 times and bring the final pH to approximately 2.

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The solutions were prepared so that the desired dose was contained in 2 ml., and the final pH was about 2. The stomach of a rat weighing 100 to 200 Gm. easily contains 2 ml. of fluid. The pH of its contents is less than 2 in both iron-supplemented and iron-deficient rats. Food was withdrawn 4 hours before dosing. Under light ether anesthesia a gum-elastic or soft rubber catheter was passed into the stomach and 2 ml. dosing solution delivered from a syringe. Each rat was put in a glass metabolic cage and food was replaced one hour after dosing. Feces and urine were collected together for 12 days. The percentage of dose retained and presumed to have been absorbed was measured by the following three methods:

1. Direct counting of collected feces—The feces and urine were counted in the glass jar used for collection, placed in a large well-type phosphor scintillation counter. The well measures 12.8 cm. deep by 12.0 cm. in diameter internally. Its sensitivity for Fe59 is sufficiently uniform that the counting rates for a given amount at volumes from 10 to 400 ml. do not differ by more than ± 2.5 per cent from the counting rate at 200 ml. Thus, samples and standard solutions of different volumes may be compared directly.

2. Counting aliquots of feces—The radioactivity of aliquots of mixed feces was measured by means of a conventional sodium-iodide surface scintillation counter.

3. Whole body counting—On the 12th day after dosing, the rat was placed in a waxed cardboard carton with a perforated lid and its radioactivity measured in the phosphor well counter. In this apparatus continuous serial counts on rats in the first few hours after administration of the dose do not differ by more than ± 3 per cent from the mean counting rate despite movement of the rat and redistribution of the dose within its body.

Results of measuring percentage of dose in a series of fecal collections, (1) by direct counting of collection and (2) by counting aliquots, are compared in fig. 1. Correlation is good (r = 0.985). Discrepancies are greater when over 90 per cent of the dose is present in the collection, and are more often in the direction of a lower result from method 2. This may in part be due to small losses of material in transferring collections from collecting jars for mixing. Methods (1) and (3) are also compared in fig. 1. Correlation is very good (r = -0.995).

Results

General, Hematologic and Histologic Findings

At the time of testing iron absorption, the iron-deficient rats weighed 100 to 200 Gm., while their iron-supplemented litter-mates weighed 150 to 250 Gm. Histograms show weights (fig. 2) and hemoglobin levels (fig. 3) at the time of testing. On the usual dietary regime (diet 1A followed by 1B), the hemoglobin levels of the iron-deficient rats fell to 5 to 6 Gm. per 100 ml. at 3 months of age, then gradually rose (on diet 1B) to 8 to 10 Gm. per 100 ml. at 10 months of age. The apparent bimodality of hemoglobin levels in the iron-deficient (1B) group, at the time of testing, is an artificial appearance due to the fact that absorption tests have tended to be clumped at 4 and 6 months. The average hemoglobin levels and M.C.H.C. for the iron-deficient (1B) group were, respectively, 6.9 Gm. per 100 ml. and 27.0 per cent, and for the iron-supplemented group 16.2 Gm. per 100 ml. and 35.7 per cent at the time of testing.

Iron-deficient rats maintained throughout on diet 1A had hemoglobin levels which were slightly lower than those of rats on diet 1B, but showed the same general pattern of a fall during rapid growth, then a leveling off, and finally a slight rise to about 6 Gm. per 100 ml. at 5 months of age. Adult male rats on standard rat-cake diet had hemoglobin levels of 15.5 to 17.5 Gm. per 100 ml.
Fig. 1.—Correlation between methods of measuring iron absorption: Left, relationship between percentage of dose of radioactivity present in 12-day fecal collection as determined by method (1), direct counting of whole collection in phosphor well counter, and by method (2) counting aliquots of mixed collection on a surface scintillation counter \((r = 0.985)\). The regression line \(y = 2.091 + 0.939x\) is shown; the alternative line \(x = 1.0347y - 0.627\) is almost identical. Right, relationship between percentage of dose \([\text{method (1)}]\), present in 12-day fecal collection, and method (3) remaining in rat at 12th day, both determined by direct counting in phosphor well counter \((r = -0.995)\). The regression line \(y = 96.90 - 0.96x\) is shown; the alternative line \(x = 100.665 - 1.036y\) is almost identical.

The serum iron levels for rats on diet 1B were 35 to 68 \(\mu\)g per 100 ml. (mean of 8 values, 49.12 \(\pm\) 12.44) and for diet 2 iron-supplemented rats, 152 to 500 \(\mu\)g per 100 ml. (mean of 6 values 287.3 \(\pm\) 134.6).

Rate of Elimination

The rapidity with which the unabsorbed portion of the dose is eliminated in the feces is an indication of the rate of transit of the Fe59 through the alimentary tract after it has been put in the stomach. Observations on the

Fig. 2.—Histograms showing the distribution of body weights of female iron-deficient (diet 1B) and iron-supplemented (diet 2) litter mate rats at the time of testing in the main series of tests.
rate of elimination were made during absorption tests on four rats on standard cake diet, 14 rats on iron-supplemented diet 2 and 11 iron-deficient rats on diets 1A and 1B. After intragastric administration of test doses of Fe\textsuperscript{59}, feces were collected and their radioactivity measured and whole body radioactivity was usually measured also, at intervals of 1 to 4 days up to a maximum of 30 days. About 95 per cent of the total radioactivity excreted up to 30 days by rats fed on rat cake or diet 2 appears in the feces within the first 4 days, much of it in the first 48 hours. In iron-deficient rats, however, sometimes little appears in the first 48 hours and although over 50 per cent of the total may appear within 4 days, significant quantities appear up to the 12th day and thereafter. Examples of this characteristic difference in pattern of elimination are shown in figure 4. The patterns were the same when the rats were not starved before Fe\textsuperscript{59} dosing.

It was concluded that the rate of intestinal transit of a dose of Fe\textsuperscript{59} under these conditions is slower in iron-deficient than in control rats. This conclusion is of practical importance in experiments for measuring intestinal absorption, since a period of collection or observation adequate for measuring absorption in iron-supplemented rats may be too short for iron-deficient rats. On the other hand, there are disadvantages in an unduly long collection period, as it is likely that part of the radioactivity appearing in rats' feces after oral
Fig. 4.—The patterns of elimination of Fe\textsuperscript{59} in the feces following an intragastric dose of 50 μg. Fe. Results are shown from two iron-deficient rats (304 and 320) and their iron-supplemented litter mates (303 and 318). Blocks represent on a logarithmic scale, the percentage of the dose eliminated per day for each collection period. Collections were continued for 30 days from dosing; after the 6th (318) or 12th (303) day, the feces of the iron-supplemented rats contained less than 0.1 per cent of the dose per day.

Doses of Fe\textsuperscript{59} represent re-excretion of the absorbed portion of the dose, as discussed below. This excretion, though only a small percentage, probably continues indefinitely, so that the longer the fecal collection is continued the greater will be the error arising from the inclusion of this re-excreted fraction along with the unabsorbed fraction. For these reasons 12 days was arbitrarily chosen as the period for collection in these experiments.

Absorption of a Standard Dose (50 μg.)

Fifty μg., probably a physiologic quantity, was chosen as a standard dose. For a rat weighing 200 Gm. it represents 0.25 mg. per Kg. body weight, equivalent to a 15-mg. dose for a human subject. It is estimated that each rat consumes approximately 10 Gm. of the semisynthetic diet daily containing 40 μg. Fe (diet 1B) or 2400 μg. (diet 2). This dietary iron, however, is ingested intermittently over 24 hours and is therefore not comparable with a single dose.

Absorption of 50 μg. Fe, given in the ferrous form as described above, was tested in 17 iron-deficient (diet 1B) and 13 iron-supplemented rats at different times during the period of tests. The results were closely similar with-
Table 1.—Individual Test Results for Absorption of 50 µg. Ferrous Iron by Iron-deficient and Iron-supplemented Rats

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Age in Days</th>
<th>Weight (Gm.)</th>
<th>Hemoglobin (Gm./100 ml.)</th>
<th>M.C.H.C.</th>
<th>Per cent Absorption</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Iron-Deficient Group (Diet 1B)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mean values ± S.D.</td>
</tr>
<tr>
<td>4</td>
<td>113</td>
<td>137</td>
<td>4.0</td>
<td>—</td>
<td>166 ± 7.5</td>
</tr>
<tr>
<td>8</td>
<td>113</td>
<td>120</td>
<td>4.3</td>
<td>—</td>
<td>6.6 ± 1.8</td>
</tr>
<tr>
<td>106</td>
<td>124</td>
<td>173</td>
<td>7.1</td>
<td>27.3</td>
<td>26.9 ± 4.0</td>
</tr>
<tr>
<td>108</td>
<td>124</td>
<td>193</td>
<td>7.7</td>
<td>—</td>
<td>88.7 ± 7.2</td>
</tr>
<tr>
<td>116</td>
<td>124</td>
<td>165</td>
<td>5.5</td>
<td>—</td>
<td>82.7 ± 7.2</td>
</tr>
<tr>
<td>203</td>
<td>139</td>
<td>163</td>
<td>6.7</td>
<td>35.3</td>
<td>81.7 ± 7.2</td>
</tr>
<tr>
<td>212</td>
<td>139</td>
<td>154</td>
<td>4.2</td>
<td>23.3</td>
<td>76.7 ± 7.2</td>
</tr>
<tr>
<td>213</td>
<td>139</td>
<td>156</td>
<td>6.8</td>
<td>25.2</td>
<td>71.7 ± 7.2</td>
</tr>
<tr>
<td>216</td>
<td>139</td>
<td>148</td>
<td>5.2</td>
<td>22.6</td>
<td>85.7 ± 7.2</td>
</tr>
<tr>
<td>1</td>
<td>161</td>
<td>156</td>
<td>10.4</td>
<td>28.9</td>
<td>88.7 ± 7.2</td>
</tr>
<tr>
<td>9</td>
<td>161</td>
<td>184</td>
<td>5.5</td>
<td>22.9</td>
<td>91.7 ± 7.2</td>
</tr>
<tr>
<td>11</td>
<td>161</td>
<td>217</td>
<td>7.6</td>
<td>26.2</td>
<td>89.7 ± 7.2</td>
</tr>
<tr>
<td>19</td>
<td>161</td>
<td>158</td>
<td>9.0</td>
<td>33.3</td>
<td>85.7 ± 7.2</td>
</tr>
<tr>
<td>20</td>
<td>161</td>
<td>161</td>
<td>5.4</td>
<td>23.5</td>
<td>81.7 ± 7.2</td>
</tr>
<tr>
<td>4</td>
<td>186</td>
<td>190</td>
<td>7.4</td>
<td>26.4</td>
<td>68.7 ± 7.2</td>
</tr>
<tr>
<td>8</td>
<td>186</td>
<td>172</td>
<td>8.2</td>
<td>27.3</td>
<td>86.7 ± 7.2</td>
</tr>
<tr>
<td>7</td>
<td>245</td>
<td>141</td>
<td>7.4</td>
<td>—</td>
<td>82.7 ± 7.2</td>
</tr>
</tbody>
</table>

Mean values ± S.D. | 207 ± 21.5 | 16.3 ± 0.9 | 35.7 ± 2.0 | 7.9 ± 5.7 |

|         |             |              |                          |           | Iron-supplemented Group (Diet 2) |
|         |             |              |                          |           | Mean values ± S.D. |
| 24      | 113         | 189          | 17.2                     | —         | 18.7 ± 3.2 |
| 38      | 113         | 165          | 16.6                     | —         | 20.7 ± 3.2 |
| 223     | 139         | 194          | 16.6                     | 34.6      | 8.7 ± 1.2   |
| 233     | 139         | 207          | 15.7                     | 35.7      | 6.7 ± 1.2   |
| 236     | 139         | 229          | 15.4                     | 40.5      | 10.7 ± 1.2  |
| 282     | 139         | 202          | 16.8                     | 34.5      | 5.7 ± 1.2   |
| 24      | 186         | 234          | 17.2                     | 37.4      | 3.7 ± 1.2   |
| 30      | 186         | 223          | 16.9                     | —         | 8.7 ± 1.2   |
| 38      | 186         | 194          | 16.4                     | 36.4      | 3.7 ± 1.2   |
| 39      | 186         | 219          | 14.2                     | 33.8      | 6.7 ± 1.2   |
| 40      | 186         | 182          | 17.2                     | 35.1      | 0.7 ± 1.2   |
| 33      | 245         | 221          | 15.4                     | 34.2      | 5.7 ± 1.2   |
| 35      | 253         | 233          | 16.9                     | 34.5      | 10.7 ± 1.2  |

Tests were carried out at different times during the period of study, so that ages, weights, hemoglobin levels and M.C.H.C. vary as shown. Repeated results on the same animals are included. The absorption results given here were those obtained by method (2), counting aliquots of mixed fecal collections expressed to the nearest one per cent.

in each group (table 1). The mean percentage absorption for iron-deficient rats was 82.7 per cent and for iron-supplemented rats 7.9 per cent. Within each group no close correlation can be discerned between percentage absorption and individual hemoglobin level, M.C.H.C., body weight, or age at the time of testing, though the total numbers are of course small. Thus, although anemic (and smaller) rats absorbed on an average 10 times as much as their non-
Table 2.—Absorption of 50 μg. Ferrous or Ferric Iron by Groups of Rats on Different Diets

<table>
<thead>
<tr>
<th>Rats</th>
<th>Number Tested</th>
<th>Diet</th>
<th>Iron Content of Diet (pg. Fe/10 Gm. Diet)</th>
<th>Mean Body Weight (Gm.)</th>
<th>Mean Hb (Gm./100 ml.)</th>
<th>Per cent Absorption, Mean ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young female</td>
<td>3</td>
<td>1A</td>
<td>20</td>
<td>50 pg. Fe²⁺</td>
<td>169</td>
<td>4.3</td>
</tr>
<tr>
<td>Young females, litter mates</td>
<td>3</td>
<td>1B</td>
<td>40</td>
<td>50 pg. Fe²⁺</td>
<td>178</td>
<td>6.2</td>
</tr>
<tr>
<td>Young females, group (table 1)</td>
<td>17</td>
<td>1B</td>
<td>40</td>
<td>50 pg. Fe²⁺</td>
<td>166</td>
<td>6.6</td>
</tr>
<tr>
<td>Young females, group (table 1)</td>
<td>13</td>
<td>2</td>
<td>2400</td>
<td>50 pg. Fe²⁺</td>
<td>207</td>
<td>16.3</td>
</tr>
<tr>
<td>Adult males, group</td>
<td>6</td>
<td>Rat cake</td>
<td>950</td>
<td>50 pg. Fe²⁺</td>
<td>488</td>
<td>16.4</td>
</tr>
<tr>
<td>Young female, litter mates</td>
<td>4</td>
<td>1A</td>
<td>20</td>
<td>50 pg. Fe²⁺</td>
<td>165</td>
<td>5.8</td>
</tr>
</tbody>
</table>

Table 3.—Absorption of Doses of Ferrous Iron of Different Sizes by Iron-deficient and Iron-supplemented Rats

<table>
<thead>
<tr>
<th>Dose (μg. Fe²⁺)</th>
<th>No. of Rats</th>
<th>Per cent Absorption, Mean ± S.D.</th>
<th>μg. Absorbed, Mean</th>
<th>No. of Rats</th>
<th>Per cent Absorption, Mean ± S.D.</th>
<th>μg. Absorbed, Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>3</td>
<td>79.6 ± 4.7</td>
<td>4.0</td>
<td>4</td>
<td>26.5 ± 14.2</td>
<td>1.3</td>
</tr>
<tr>
<td>12.5</td>
<td>4</td>
<td>86.3 ± 0.5</td>
<td>10.8</td>
<td>4</td>
<td>21.3 ± 14.4</td>
<td>2.7</td>
</tr>
<tr>
<td>25</td>
<td>4</td>
<td>86.8 ± 3.6</td>
<td>21.7</td>
<td>4</td>
<td>17.0 ± 17.8</td>
<td>4.3</td>
</tr>
<tr>
<td>50</td>
<td>17</td>
<td>82.7 ± 7.2</td>
<td>41.3</td>
<td>13</td>
<td>7.9 ± 5.7</td>
<td>3.9</td>
</tr>
<tr>
<td>100</td>
<td>3</td>
<td>78.0 ± 3.4</td>
<td>78.0</td>
<td>3</td>
<td>9.0 ± 13.0</td>
<td>9.0</td>
</tr>
<tr>
<td>150</td>
<td>3</td>
<td>80.0 ± 5.5</td>
<td>120.0</td>
<td>3</td>
<td>10.3 ± 9.0</td>
<td>15.5</td>
</tr>
<tr>
<td>200</td>
<td>5</td>
<td>61.0 ± 12.1</td>
<td>122.0</td>
<td>5</td>
<td>7.2 ± 3.4</td>
<td>14.4</td>
</tr>
<tr>
<td>350</td>
<td>6</td>
<td>78.2 ± 5.8</td>
<td>273.7</td>
<td>3</td>
<td>7.3 ± 2.4</td>
<td>23.5</td>
</tr>
<tr>
<td>500</td>
<td>4</td>
<td>51.8 ± 4.1</td>
<td>258.8</td>
<td>4</td>
<td>10.0 ± 8.7</td>
<td>50.0</td>
</tr>
<tr>
<td>1,000</td>
<td>4</td>
<td>65.3 ± 4.2</td>
<td>653.3</td>
<td>4</td>
<td>4.8 ± 1.9</td>
<td>48.0</td>
</tr>
</tbody>
</table>

Values given here are those determined by method (2) using aliquots of mixed fecal collections, except for the 1,000 μg. dose where method (1) only was used.

anemic larger sisters, among the whole group of anemic animals on diet 1B there was no evidence that the most anemic absorbed more than the least anemic or that absorption was greater in the younger rats. However, a small group of more anemic rats maintained throughout on diet 1A shows a slightly higher level of absorption than their litter mates maintained on diet 1B (table 2) so that the results for the diet 1B group do not necessarily represent the maximum absorption attainable by iron-deficient rats.

Full-grown normal adult male rats maintained on standard rat cake absorbed an average of 13.6 per cent of 50 μg. ferrous doses.

When the 50 μg. dose was given as ferric iron (ferric chloride solution), the percentages absorbed by iron-deficient (diet 1A) and iron-supplemented (diet 2) rats were similar to the percentages of ferrous iron absorbed (table 2).

Absorption of Increasing Doses of Ferrous Iron

The results of a series of tests of absorption using method (2) are shown in table 3 and figure 5. The weights and hemoglobin levels in the rats at the time...
of testing are shown in figures 2 and 3. In each test iron-deficient rats were usually compared with their iron-supplemented litter mates, but the results have here been combined for convenience.

Greater absorption by the iron-deficient rats was found at all doses tested and was about 80 per cent of doses up to 150 \( \mu g \). (fig. 5). For these animals the relationship between size of dose and amount absorbed is a direct linear one, certainly in the dose range 25 to 150 \( \mu g \), and probably up to 1,000 \( \mu g \). (fig. 6). There is no indication of a limiting level up to this dose. Iron-supplemented rats absorbed a much smaller percentage of the same doses but each increase in size of dose up to 500 \( \mu g \), was associated with an increase in the absolute quantity absorbed. The relationship between size of dose and amount absorbed is again a linear one in the range from 25 to 200 \( \mu g \). However, for both groups the relationship appears curvilinear at the lower end of the dose scale (fig. 6).

*The Effects of Switching Diet*

In two experiments, small groups of four to 12 rats which had been maintained on either iron-deficient diets or iron-supplemented diets were switched over, so that those previously taking the iron-supplemented diet 2 were switched to the iron-deficient diet 1A, and those on the deficient diets 1A or 1B were switched to 2. Absorption was tested in single rats taken from each group at intervals from one day onward by measuring the absorption of 50
Fig. 6.—Absorption of increasing doses of ferrous iron by iron-deficient (○) and iron-supplemented (●) rats, calculated from mean per cent absorption in table 3 and fig. 5. The insert shows an enlargement of the first part of the curves.

μg of iron in ferrous or ferric form. Each animal was, as usual, starved for 4 hours before and 1 hour after dosing. Preliminary experiments on diet 2 rats showed that starvation alone has little effect on per cent absorption.

The results of the two experiments are combined in figure 7. The previously deficient rats showed a rapid rise in hemoglobin, reaching the control level at about 10 days and then apparently surpassing it. Their percentage absorption fell rapidly to about the level expected on diet 2. Iron-supplemented rats put onto iron-deficient diet 1A showed no change in hemoglobin for at least the first 30 days. However, their absorption rate started to increase from the first day, reaching a level approaching that achieved by animals with long-standing deficiency.

The trend of changes of absorption, indicated by interrupted lines in the lower part of figure 7, suggests that the changes are not directly dependent upon hemoglobin level or total iron stores (see Discussion).

DISCUSSION

The simplest way of testing the absorption of an oral dose of Fe59-tagged iron is to measure directly the radioactivity retained in the body after fecal elimination is completed. It is desirable that the method of whole-body counting used should give the same results when the dose is distributed throughout the body as when it is localized, e.g., in the animal's stomach immediately after dosing or in a standard sample. The phosphor well counter used in the present investigation is a satisfactory apparatus for this purpose since variations in the position, shape and volume of the sample over a wide range have little effect on the observed counting rate. When such an apparatus is avail-
Fig. 7.—The effects of switching diet on mean hemoglobin levels (above) and on the absorption of 50 \( \mu \)g of inorganic iron (below). Composite results of two experiments: (\( \triangle \)) represents the rats switched from iron-deficient diet 1A and (\( \circ \)) those switched from iron-deficient diet 1B, to iron-supplemented diet 2. (\( \blacktriangle \)) represents iron-supplemented rats switched to iron-deficient diet 1A. Each point in the lower part of the figure is the result of testing absorption on one rat; the interrupted lines are intended to indicate the apparent trend of absorption changes.

It provides a more convenient as well as more accurate method of testing absorption in rats or other small animals by whole body counting (method 3) and in addition it may be used to count the unabsorbed portion of the dose in the whole fecal collection (method 1) without the need to homogenize and aliquot (method 2). In the present study, good correlation (\( r = -0.995 \)) was obtained between methods (1) and (3) and methods (1) and (2) (\( r = 0.985 \)). This compares favourably with results of Field et al.\(^{20}\) using a sodium-iodide surface scintillation counter (\( r = 0.969 \), calculated from their published results). Although results from method (2) have been used for many of the experiments reported here, because the phosphor well counter was not available when they were started, this counter has been used for all subsequent experiments. Whole-body counting is well adapted for carrying...
out measurements on a large number of animals as individual metabolic cages are not needed.

The iron-deficient rats are assumed to have been suffering from an uncomplicated state of iron deficiency. In iron-deficiency anemia in man, nonspecific gastrointestinal symptoms are not uncommon, and may subside with treatment, but delay in intestinal transit has not to our knowledge been described. In the iron-deficient rats on iron-deficient diet, the possibility of slower transit is suggested by the observed delay in elimination of the unab- sorbed portion of the dose. This finding confirms the results of early radioactive-iron studies made with large doses of iron in acute experiments by Austoni and Greenberg and Copp and Greenberg. These authors suggested that the delay occurred in the large intestine. It has been found in this laboratory that the ceca of the iron-deficient rats are consistently enlarged, apparently by passive distension and although the mechanism of delayed elimination is unknown, one hypothesis would be that it is due to retention of fecal material in the cecum. Alternatively, cecal distension might be a manifestation of a more general alteration of intestinal motility leading to delay in transit.

To interpret the present results a distinction must be made between radioactivity due to elimination of unabsorbed iron and that due to re-excretion following absorption. Fecal excretion is observed after intravenous or intraperitoneal doses of Fe and excretion by this route in the rat is probably of importance in the total iron balance. Inspection of figure 4 shows that the two fractions can be distinguished easily in iron-supplemented rats but less easily in the iron-deficient rats.

The striking enhancement of absorption of inorganic iron by rats with iron-deficiency anemia confirms previous observations. There is, for instance, a tenfold difference in the absorption of a 50 μg. dose. Iron-deficient rats absorbed more than iron-supplemented litter mates at all doses tested. How iron deficiency enhances absorption is still unknown. The differences between the two groups of rats can be tabulated as follows:

<table>
<thead>
<tr>
<th>Factor</th>
<th>Fe Deficient</th>
<th>Fe Supplemented</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Hemoglobin (means)</td>
<td>6.9 Gm./100 ml.</td>
<td>16.2 Gm./100 ml.</td>
</tr>
<tr>
<td>2. M.C.H.C. (means)</td>
<td>27.0 per cent</td>
<td>35.7 per cent</td>
</tr>
<tr>
<td>3. Serum iron (ranges)</td>
<td>35-68 μg./100 ml.</td>
<td>152-500 μg./100 ml.</td>
</tr>
<tr>
<td>4. Intestinal iron staining²⁷</td>
<td>absent</td>
<td>present</td>
</tr>
<tr>
<td>5. Iron stores*</td>
<td>reduced</td>
<td>normal or increased</td>
</tr>
<tr>
<td>6. Dietary iron</td>
<td>4 mg./Kg. diet</td>
<td>240 mg./Kg. diet</td>
</tr>
<tr>
<td>7. Erythropoietic stimulus*</td>
<td>? increased</td>
<td>normal</td>
</tr>
<tr>
<td>8. Intestinal motility</td>
<td>? reduced</td>
<td>normal</td>
</tr>
<tr>
<td>9. Body weight</td>
<td>150-200 Gm.</td>
<td>200-250 Gm.</td>
</tr>
</tbody>
</table>

*See McCall and McCall et al.

It is impossible to decide which of the factors listed above are most significant in determining increased absorption, and it is likely that it depends upon several. Indeed it may be impossible to decide how iron deficiency influences iron absorption until the normal mechanism of absorption is understood. The effects of switching diets in the present experiments direct attention to
local factors in the intestine. Since the iron-supplemented rats switched to
iron-deficient diet show an immediate increase in absorption when hemoglobin
level has not altered and before total body stores are likely to have changed,
a local factor, such as the amount of iron in the intestinal tissues, must be of
greater importance. The reverse diet-switching experiments can be interpreted
in the same way, since when absorption falls, although hemoglobin level is
rising towards normal, body iron stores are unlikely to have become filled,
and again local factors must be of importance.

For a series of doses of inorganic iron of increasing size, the more iron is
given the more is absorbed, in normal rats28 and in normal and iron-deficient
human subjects29 and mice.30 In the present study, there is a straight line
relationship between size of dose and amount absorbed over a large part of
the dose range for both iron-supplemented and iron-deficient rats. At the
lower part of the range the relationship is curvilinear (fig. 6). These findings
can be viewed in terms of two processes: 1) simple diffusion, to explain the
direct linear relationship, particularly in the iron-deficient rats; and 2) a car-
rier mechanism. Similar conclusions have been reached and mathematical
expressions provided by Gitlin and Cruchaud in their work with mice.30

A straight-line relationship on a log/log scale between the amount of iron
absorbed and the amount of iron present in isolated intestinal loops in vivo
has been noted by Duthie, Code and Owen.31 Our results plotted in this way
also give straight lines for both groups of rats. The significance of this relation-
ship is not clear, but it is of interest that it may hold also for iron administered
in an organic form, namely Fe59-tagged hemoglobin, according to preliminary
experiments in this department.

Ferrous and ferric iron were equally well absorbed at the standard dose
tested (50 μg.), confirming some previous reports21,32 which indicated that the
valency form in which the iron is administered may not be of importance in
the rat. This is in contrast to the findings of Brading et al.,29 that ferric iron
is better absorbed than ferrous, but most of their comparisons were made at
doses greater than 1,000 μg.

Summary

1) Whole body counting by means of a large phosphor well scintillation
counter has been used to measure the absorption of Fe59-tagged inorganic iron,
and shown to compare favourably with other methods.

2) There is a delay in the fecal elimination of the unabsorbed portion of
the dose of Fe59 by iron-deficient rats on iron-deficient diet. The cause of this
delay is unknown but it may be associated with the marked cecal enlargement
which exists in these animals.

3) It is confirmed that iron deficiency is associated with striking enhance-
ment of absorption of ferrous and ferric inorganic iron.

4) When a series of doses of ferrous iron of increasing size from 5 to 1,000
μg. was given, there was a progressive increase in absorption for each increase
in dose in both iron-supplemented and iron-deficient rats. The relationship
between amount of iron given and amount absorbed suggests that two processes
may be involved: 1) simple diffusion, and 2) a carrier mechanism.
5) The effect on iron absorption of a sudden change in iron intake has been investigated. Switch from a low to high iron diet reduces absorption, and from a high to a low iron diet increases absorption, too rapidly for hemoglobin level or body iron stores alone to be the most important governing factors and this finding emphasizes the importance of local changes in the intestine.

SUMMARIO IN INTERLINGUA

1. Contation del corpore total, utilisante un contador de scintillation a grande puteo de phosphoro, esseva empleate pro mesurar le absorption de ferro inorganic con marcation a Fe59. Le comparation de iste metodo con alteres esseva in su favor.

2. Il occurre un retardo in le elimination fecal del non-absobite portion del dose de Fe59 in le caso de ratti a carentia de ferro que recipe un dieta que es carente in ferro. Le causa de iste retardo non es cognoscite, sed illo es possibilemente associate con le marcate allargamento del ceco que existe in iste animales.

3. Esseva confirmate le facto que carentia de ferro es associate con un frappante promotion del absorption de ferro inorganic tanto ferrose como etiam ferric.

4. Quando un serie de doses de ferro ferrose de magnitudes crescente ab 5 ad 1.000 μg esseva administrate, il occurreva un augmento progressive in le absorption con omne augmento del dose tanto in ratti a carentia de ferro como etiam in ratti recipiente supplementos de ferro. Le relation inter le quantitate de ferro administrate e le quantitate absorbite suggere que duo processos es active in le phenomeno. Illos es (1) un simple diffusion e (2) un mechanismo a vector.

5. Esseva investigate le effecto exercite super le absorption de ferro per un subite alteration in le acceptation de ferro. L transition ab un dieta a basse contento de ferro ad un dieta a alte contento de ferro reduce e le transition inverse augmenta le absorption. Iste alterationes es troppo rapide pro esser explicable per le nivello de hemoglobinina o le reservas de ferro del corpore reguardate como le plus importante factores regulatori. Le constatation servi a sublinear le importantia de alterationes local in le intestino.

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